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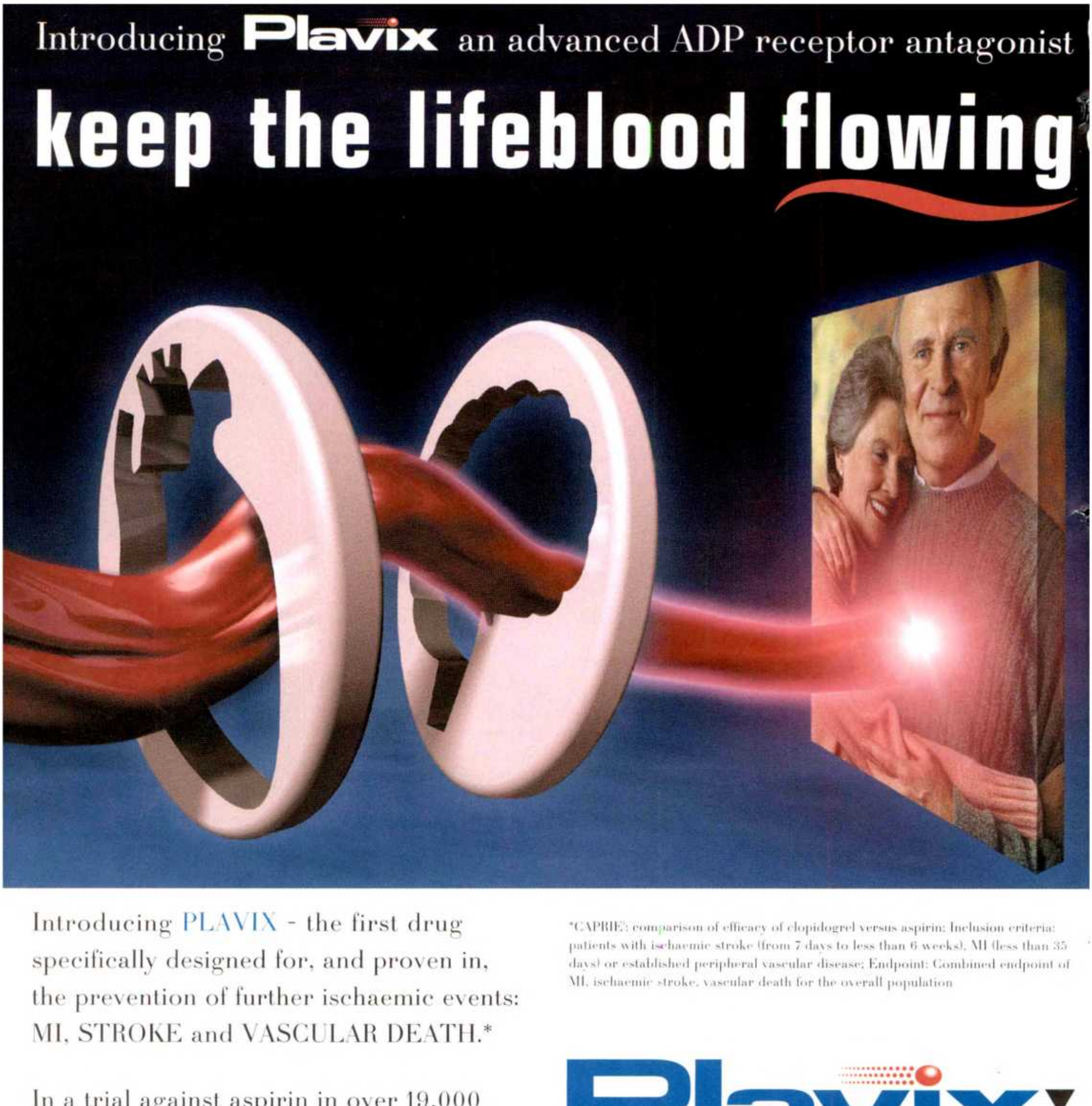
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Leading article

Stapled haemorrhoidectomy: pain or gain

F. Seow-Choen

Department of Colorectal Surgery, Singapore General Hospital, Singapore 169608 (e-mail. gcsscf@sgh.gov sg)

Introduction

Conventional scissors excision of symptomatic prolapsed haemorrhoids is a commonly performed but notoriously painful operation. Not surprisingly, the search for a painless method of haemorrhoidectomy has occupied the energies of many a surgeon. Modifications to decrease postoperative pain have included the addition of lateral internal sphincterotomy, closed haemorrhoidectomy, anal dilatation and anal sphincter relaxants, as well as the use of oral metronidazole. Although all of these techniques have had their advocates, none has achieved a sufficiently significant decrease in pain to gain universal acceptance. An exception may be diathermy haemorrhoidectomy, which seems to be associated with significantly decreased immediate postoperative discomfort. Even with this method, however, most patients still have severe pain on defaecation, and a significant number suffer from great discomfort at rest in the second and third postoperative weeks from wound infection and sphincter spasm. It is no surprise, therefore, that the introduction of stapled haemorrhoidectomy, with its claims of pain-free convalescence, has been met with great enthusiasm by some surgeons (but with cold scepticism by others).

Stapled haemorrhoidectomy, as initially advocated by Longo and colleagues, has been hailed as a significant breakthrough in the way surgical excision of haemorrhoids is performed; it both dramatically decreases the level of postoperative pain and hastens the speed of healing after operation[1]. However, some surgeons worry about leaving large skin tags behind and long-term sequelae such as strictures[2]. It seems clear, therefore, that several important questions need to be answered before this new procedure can be welcomed unreservedly. First, is postoperative pain significantly improved? Second, is the operation applicable to all patients who require surgical excision of haemorrhoids? Third, what complications are associated with the procedure? Fourth, what is the cost advantage or disadvantage of the procedure?

Pain

Two small prospective randomized trials from the UK have provided convincing evidence of decreased pain following stapled haemorrhoidectomy compared with diathermy haemorrhoidectomy[3,4]. Both studies used an analogue pain score scale. The total pain scores were significantly less for stapled *versus* diathermy operation in one study[3] ($P = 0{\cdot}003$). In the other study[4], average pain was significantly less for stapled compared with diathermy haemorrhoidectomy ($P < 0{\cdot}0001$). A larger study from Singapore[5], comprising 119 consecutive patients randomized to diathermy or stapled haemorrhoidectomy, showed that maximal pain in hospital and 2 weeks after operation, as well as pain at evacuation of stool while in hospital and 2 weeks after operation, were significantly less after stapled compared with diathermy haemorrhoidectomy. Analgesic requirements for up to 6 weeks after operation were also significantly reduced in the stapled group. At 6 and 12 weeks after operation, 15 and 5 per cent of patients with diathermy haemorrhoidectomy *versus* 5 and 2 per cent respectively of patients after stapled haemorrhoidectomy still had pain. On rectal digitation no patient had severe pain at 6 or 12 weeks after stapled haemorrhoidectomy, compared with 16 and 3 per cent respectively after diathermy haemorrhoidectomy. Patients with diathermy or scissors haemorrhoidectomy experience a severe and sharp tearing pain in the anus aggravated by, and therefore preventing, bowel movement. Pain after stapled haemorrhoidectomy is of a different sort, being vague, dull and tenesmoid in nature; this results in a need for bowel movement. Should the purse-string suture be placed too close to the dentate line, however, accidental stapling of the sensitive anoderm results in excessive postoperative pain.

A recent report from the UK described 5 patients with persistent pain after stapled haemorrhoidectomy out of a total of 22 treated[6]. This is difficult to understand as no other author has noted persistent pain after stapled haemorrhoidectomy. Indeed, a report of 206 operations with at least 6 months' follow-up in all patients failed to note any patient with persistent anal discomfort or pain[7].

Complications

Neither of the randomized UK trials[3,4] reported any major adverse side-effect for stapled haemorrhoidectomy. The Singapore study[5] showed similar total complication rates from stapled and diathermy haemorrhoidectomy, with an incidence of 18 and 26 per cent respectively (including mild strictures and bleeding). Although there was no statistically significant difference between the groups, 5 per cent of the patients having diathermy compared with none of those undergoing stapled haemorrhoidectomy had secondary haemorrhage requiring inpatient care. At 6 and 12 weeks after operation, 7 and 2 per cent of patients undergoing diathermy, and 4 and 2 per cent of those having stapled haemorrhoidectomy, had a rectal stricture. There was also no significant difference in terms of incontinence between the groups at 6 weeks and 3 months.

Other complications associated with stapling reported anecdotally have included a case of near-fatal pelvic sepsis[8] and a patient with a rectovaginal fistula[9]. The suggestion of routine prophylactic antibiotic use after all stapled haemorrhoidectomies does not seem reasonable, given the unusual nature of an isolated case of pelvic sepsis[8]. Vaginal fistula may be prevented by routine vaginal examination after closure of the stapler (before firing) to ensure that no part of the vaginal wall is accidentally included. Incidentally, patients with anoreceptive sexual practices require appropriate advice. The 'go ahead' for anal sex should be given only after rectal digitation by the surgeon has confirmed that the staple line is free of potentially dangerous sharp points. Pain and bleeding has also been reported in women as a result of anoreceptive sexual activity after stapled haemorroidectomy[6].

Applicability

Initial concern that staplers are applicable only for early haemorrhoids and not for third- or fourth-degree haemorrhoids appears to be unfounded. The concern was that the procedure amounts to overkill as smaller haemorrhoids can be treated successfully with elastic band ligation, sclerotherapy or infrared coagulation. All the published studies mentioned above involved prospective randomization only of patients with symptomatic prolapsed haemorrhoids and have compared only patients who would otherwise have needed excisional haemorrhoidectomy[3,4]. In the study from Hull[3], satisfaction with symptom control was similar in both groups, with 85 and 75 per cent in the stapled and diathermy groups respectively rating symptom control as good or excellent; one patient from the diathermy and four from the stapled group complained of persistent skin tags. In the Leicester study[4], eight of 11 diathermied patients had their symptoms controlled at 6 weeks, compared with ten of 11 in the stapled group.

In the Singapore study[5], observer-perceived residual skin tags were found in 16, 5 and 3 per cent of patients having diathermy, and in 19, 9 and 4 per cent of those undergoing stapled haemorrhoidectomy, at 2, 6 and 12 weeks after operation respectively. Thus residual skin tags after stapled haemorrhoidectomy tended to shrivel by the third month after operation. The rectal submucosal purse-string should be inserted at about 4 cm from the anal verge (not much higher) to prevent excessive residual external haemorrhoids remaining.

A further study from Singapore[10], which randomized 35 patients with thrombosed prolapsed circumferential haemorrhoids, has also shown that the stapler may be used with safety in such cases. Pain is more obvious immediately after operation following stapling in these patients, but better results are obtained in the stapled group as early as 2 weeks after operation when pain and rectal discharge are significantly less than after conventional diathermy haemorrhoidectomy.

Costs

While the PPH01® 33 stapler (Ethicon Endo-Surgery, Cincinnati, OH, USA) costs the same as an intestinal circular stapler (£230), this increased instrument cost is offset by the shorter convalescence period needed after stapled haemorrhoidectomy. Stapled haemorrhoidectomy results in minimal pain and so the procedure may be carried out in an ambulatory setting, with an earlier return to work after operation. The total cost to the community may therefore be less than that of conventional surgery. It is certainly the case that when patients are given a choice of paying the additional cost of a stapler or enduring a significantly longer period of severe pain they always opt for the stapler if it is within their financial means. Neither patient nor surgeon wants unnecessary pain and, if stapled haemorrhoidectomy is significantly less painful than other techniques, it will prove a true gain.

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Review

Systematic review of antimicrobial agents used for chronic wounds

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Background: This paper is one of a series of eight systematic reviews that aim to identify effective interventions for chronic wounds. Here, antimicrobial agents are evaluated.

Methods: Electronic databases and other relevant sources were accessed to identify published and unpublished material. Studies were eligible for inclusion if they used concurrent controls, recruited participants with chronic wounds, evaluated an intervention designed to prevent or treat chronic wounds, and incorporated an objective assessment of wound healing. All included studies were assessed against a comprehensive checklist for methodological quality. A narrative overview was conducted.

Results: Thirty trials were included, 25 of randomized design. Small sample size and other methodological problems meant that findings were often difficult to interpret. Results do not support the routine use of systemic antibiotics for leg ulcers or diabetic foot ulcers without acute infection, but they may be useful as an adjunct to surgery for pilonidal sinuses. Several topical preparations may be helpful, including dimethyl sulphoxide, silver sulphadiazine, benzoyl peroxide, oxyquinoline and gentamicin.

Conclusion: Most of this research requires replication in larger, well designed studies to establish both clinical and cost effectiveness.

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Introduction

A recent leading article in this journal explained the importance of systematic reviews of the effectiveness of health care interventions, given the difficulties in interpreting and applying evidence from small primary studies, possibly with conflicting findings[1]. A systematic review is the process of systematically locating, appraising and synthesizing evidence from scientific studies in order to obtain a reliable overview[2]. Systematic reviews are distinct from traditional narrative reviews. The latter are usually based on a selected body of literature, tend to reflect the author's beliefs and attitudes, and are therefore prone to bias. Systematic reviews, on the other hand, incorporate comprehensive literature searches, predefined selection criteria for primary material and quality assessment of individual studies. They incorporate a rigorous system of double checking of decisions and data extraction to enable a relatively objective estimate of treatment effect in an explicit and transparent manner.

This paper reviews the evidence of the clinical and cost effectiveness of systemic and topical antimicrobial agents used for chronic wounds. It is one of a series of eight systematic reviews of wound care, commissioned by the UK National Health Service (NHS) Health Technology Assessment Programme.

Methods

Search strategy

Nineteen electronic databases, including Medline and the Cumulative Index to Nursing and Allied Health Literature (CINAHL), were searched from inception to January 2000, with no restriction on date or language. Relevant journals, conference proceedings and bibliographies of retrieved articles were hand searched, and an expert panel was consulted. The expert panel included specialists in surgery, general medicine, dermatology, tissue viability nursing and wound healing research. Both published and unpublished reports were sought. Further details are available direct from the authors and from the worldwide web (http://www.hta.nhsweb.nhs.uk/).

Inclusion criteria

Studies were eligible for inclusion if they satisfied the following criteria.

1. A randomized or non-randomized design with concurrent control group, with patients, limbs or lesions as the units of allocation.
2. Participants had, or were at risk of developing, diabetic foot ulcers, pressure ulcers, chronic leg ulcers, pilonidal sinuses, non-healing surgical wounds or chronic cavity wounds.
3. The intervention consisted of healing by secondary intention using systemic or topical antimicrobial preparations, including antibiotics, antifungal and antiviral agents, or was intended to prevent the formation of chronic wounds.
4. An objective assessment of wound healing (change in ulcer size, rate of healing, complete healing, time to healing) was used. For prevention studies, change in skin condition or development of new lesions had to be reported.

Quality assessment

All included studies were assessed against a comprehensive checklist for methodological quality.

Review process

Study selection, data extraction and validity assessment were performed independently by two reviewers, and disagreements were resolved through discussion. For each study, odds ratios (ORs) and effect sizes (ESs), with associated 95 per cent confidence intervals (CI), were estimated for dichotomous and continuous data, respectively, on an intention-to-treat basis where possible. It was not possible to produce estimates for all outcomes owing to a lack of reported data. For two studies, it was not possible to produce estimates of treatment effect for any of the reported outcomes[3,4]. *Tables 1–9* show brief data extraction details of included studies, with results as reported in the original papers. Data extraction tables are available on the worldwide web (http://www.hta.nhsweb.nhs.uk/).

Data synthesis (combining results from groups of studies)

A narrative overview of the studies was conducted, with results grouped according to wound type. Statistical pooling of results from trials was considered to be inappropriate owing to differences between studies with respect to one or more important variables, e.g. characteristics of participants, interventions or outcomes.

Results

Literature search

Thirty studies met the review criteria, of which 25 were randomized controlled trials (RCTs) (J. Vandeputte and L. Gryson, unpublished report)[4–6,8,9,11–16,18–28,30,31] and five were non-randomized[3,7,10,17,29].

Assessment of methodological quality

Around half of the included studies comprised treatment arms of 20 patients or fewer (J. Vandeputte and L. Gryson, unpublished report)[3,4,6,7,9,11,14,16–19,24,29]. Other problems included failure to describe method of randomization (or other method of treatment allocation in the case of non-randomized trials)[3,4,6,9–12,14–23,26,28,31], unclear or no selection criteria for participants[3,4,7,15–17,19,27,29–31], insufficient details of baseline group comparability[4,6,10,16,17,19,22–24,27,29], lack of blinded outcome assessment (J. Vandeputte and L. Gryson, unpublished report)[3–5,7,11,13,14,17–19,23,24,27–31], lack of data on withdrawals[9,22,29,30], and failure to analyse on an intention-to-treat basis[5,6,9,10–12,16,20–23,25,26,28–30]. Further tabulated details are available from the worldwide web (http://www.hta.nhsweb.nhs.uk/).

Venous leg ulcers: systemic agents: two trials (*Table 1*)

One RCT assessed the effect of adding antibiotics, prescribed according to baseline sensitivities, to a topical regimen that included compression bandaging[5]. Patients were excluded if there were clinical signs of wound infection and/or negative bacterial wound surface cultures at baseline. There were no statistically significant differences in ulcer healing rates or frequency of complete healing after 20 days: ES 4·40 (95 per cent CI – 11·64 to 20·44) and OR 0·54 (95 per cent CI 0·15–1·98) respectively. A second evaluation compared ciprofloxacin, trimethoprim and placebo; all were used with zinc ointment and an elastic bandage[6]. Although presence of clinical infection or baseline bacterial colonization of the wound were not mentioned as a selection criterion for participants, ulcer cultures and sensitivities were assessed at baseline and at intervals of 4 weeks during the 12-week regimen. No statistically significant differences between groups were reported at 16 weeks for complete healing or change in ulcer area. The

Table 1 Venous leg ulcers: systemic agents

Reference	Year	Methods	Participants	Interventions	Outcomes	Adverse effects and other notes
Alinovi *et al.*[5]	1986	RCT with *a priori* power calculation Outcomes assessed at 20 days	48 patients with venous leg ulcers	1. Co-trimoxazole, gentamicin or amikacin given according to baseline sensitivities (cultures taken from wound surface) 2. No antibiotics All patients received pressure bandaging	Healing rate (%)* 1. 61·6 (25·8) 2. 57·2 (29·3) (n.s.) Complete healing† 1. 5 of 30 (17) 2. 7 of 26 (27) (n.s.)	
Huovinen *et al.*[6]	1994	Double-blind RCT Outcomes assessed at 16 weeks (drugs given for 12 weeks)	36 patients with venous leg ulcers of longer than 3 months' duration	1. Ciprofloxacin 750 mg p.o. b.d. 2. Trimethoprim 160 mg p.o. b.d. 3. Placebo p.o. b.d. All dressings secured with support bandage	Complete healing† 1. 5 of 12 (42) 2. 3 of 9 (33) 3. 3 of 10 (30) (n.s.) Change in ulcer size (%)‡ 1. −75 (+15 to −100) 2. −35 (+70 to −100) 3. −70 (+35 to −100) (n.s.)	This was a pilot study. No follow-up evaluation was identified

Values are *mean(s.d.); †values in parentheses are percentages; ‡values are average (range). RCT, randomized controlled trial; p.o., oral route; b.d., twice daily; n.s., not significant

ORs for complete healing were 2·14 (95 per cent CI 0·38–12·20) for both ciprofloxacin *versus* trimethoprim and ciprofloxacin *versus* placebo, and 1·00 (95 per cent CI 0·16–6·35) for trimethoprim *versus* placebo.

Venous leg ulcers: topical agents: seven trials

(*Table 2*)

The addition of polynoxylin paste to lint dressing was compared with lint dressing alone in women receiving compression therapy and injected sclerosant agents[7]. No details were given about whether clinical infection or bacterial colonization of the wound influenced selection of participants. Analysis was based on 17 patient pairs matched for baseline wound area. No statistically significant differences between groups were reported for time to healing or healing rate: ES for healing rate –0·30 (95 per cent CI – 2·05 to 1·45).

Dimethyl sulphoxide (DMSO) was compared with allopurinol and placebo in patients receiving compression bandaging[8]; DMSO is thought to have antimicrobial properties[32]. The presence of signs of clinical infection (criteria for diagnosis not defined) was an exclusion criterion for participants. There was no difference between the two active drugs at 3 months, but both achieved significantly higher rates of complete healing and greater reduction in ulcer area compared with placebo. Mean(s.e.m.) ulcer area remaining at 3 months for DMSO *versus* placebo: 0·2(0·1) *versus* 1·3(0·3) cm^2, ES 1·10 (95 per cent CI 0·46–1·74); for DMSO *versus* allopurinol: 0·2(0·1) *versus* 0·3(0·1) cm^2, ES 0·10 (95 per cent CI – 0·18 to 0·38). For complete healing, the values were 95 *versus* 70 per cent, OR 10·67 (95 per cent CI 2·30–49·39); and 95 *versus* 93 per cent, OR 2·04 (95 per cent CI 0·36–11·69) respectively.

Another study compared the application of 2 per cent mupirocin tulle gras with vehicle only[9]. Both groups received compression therapy. No statistically significant differences were reported at 12 weeks for change in ulcer area or rates of complete healing; OR for the latter was 1·31 (95 per cent CI 0·31–5·49). Presence of clinical infection and/or wound colonization were not mentioned as selection criteria for participants.

A fourth study assessed the effect of povidone–iodine solution used with hydrocolloid compared with hydrocolloid alone, secured with elastic stockings[10]. This was a non-randomized controlled trial in which patients had at least two leg ulcers and acted as their own controls. There was no statistically significant between-group difference in healing index at 8 weeks: ES 1·00 (95 per cent CI – 0·37 to 2·37). Presence of clinical infection and/or wound colonization were not described as participant selection criteria.

Table 2 Venous leg ulcers: topical agents

Reference	Year	Methods	Participants	Interventions	Outcomes	Adverse effects and other notes
Pegum and Fegan[7]	1968	Non-randomized trial (alternate allocation)	34 female patients with venous leg ulcers	1. Polynoxylin paste applied and covered with sterile lint 2. Sterile lint only All patients received compression sclerotherapy	Healing rate (mm^2/day)* 1. 2·8(2·3) 2. 3·1(2·7) (n.s.) Mean time to healing (days) 1. 37 2. 34 (Significance not reported)	
Salim[8]	1991	Double-blind RCT with *a priori* power calculation Outcomes assessed at 12 weeks	153 patients with primary venous ulceration and and baseline wound area less than 10 cm^2	1. Allopurinol powder 2. DMSO powder 3. Placebo powder All patients received compression bandaging	Complete healing (%) 1. 93 2. 95 3. 70 ($P < 0·01$ 1 *versus* 3 and 2 *versus* 3) Ulcer area (cm^2)† 1. 0·3(0·1) 2. 0·2(0·1) 3. 1·3(0·3) ($P < 0·01$ 1 *versus* 3 and 2 *versus* 3)	Four patients withdrew from the study due to local irritation (allopurinol 1, DMSO 2, placebo 1)
Cameron *et al.*[9]	1991	Double-blind RCT Outcomes assessed at 12 weeks	30 patients with venous leg ulcers and an ankle pressure index greater than 0·8 measured by Doppler	1. 2% calcium mupirocin tulle gras 2. Vehicle of above All patients received compression bandaging	Change in ulcer area (%)‡ 1. −50 (+55 to −100) 2. −68 (−35 to −100) (n.s.) Complete healing (%) 1. 53 2. 46 (n.s.)	
Pierard-Franchimont *et al.*[10]	1997	Non-randomized trial All patients had at least 2 ulcers and acted as their own controls Outcomes assessed at 8 weeks	42 female patients with at least 2 venous leg ulcers, with area within the range 12–50 cm^2	1. Povidone–iodine 10% applied to wounds covered with hydrocolloid dressing, secured with elastic bandage 2. As above, without povidone–iodine	Healing index§ 1. 10·3 (9·3–11·3) 2. 9·3 (8·3–10·3) (n.s.)	Although the authors of this study report outcomes in terms of median(95% c.i.) it is possible that they intended to report median (range)

Table 2 Continued

Reference	Year	Methods	Participants	Interventions	Outcomes	Adverse effects and other notes
Wunderlich and Orfanos[11]	1991	RCT Complete healing assessed at 6 weeks Reduction in ulcer area assessed at 2, 4 and 6 weeks	40 patients with venous leg ulcers	1. Silver-impregnated activated charcoal dressing used for all stages of wound healing 2. Various topical agents used for different stages of wound healing All wounds were debrided	Complete healing¶ 1. 6 of 19 (32) 2. 2 of 19 (11) (n.s.) Average reduction in ulcer area (%) 2 weeks 1. 40 2. 15 ($P < 0.05$) 4 weeks 1. 60 2. 40 ($P < 0.05$) 6 weeks 1. 75 2. 60 (n.s.)	
Bishop *et al.*[12]	1992	RCT, 2 centres Outcomes assessed at 4 weeks	90 patients with venous leg ulcers of at least 3 months' duration, surface area 3–50 cm^2 with < 10^5 bacteria per g ulcer tissue	1. Tripeptide–copper complex 0·4% 2. Silver sulphadiazine 1% cream 3. Placebo All dressings secured with elastic bandage	Decrease in ulcer area(%)* 1. 18·7(9·1) 2. 44·0(8·2) 3. 22·5(10·2) ($P = 0.03$ for 1 *versus* 2, $P = 0.05$ for 2 *versus* 3, n.s. 1 *versus* 3) Complete healing¶ 1. 0 of 29 (0) 2. 6 of 28 (21) 3. 1 of 29 (3)	
Blair *et al.*[13]	1988	RCT Outcomes assessed at 12 weeks	60 patients with venous leg ulcers up to 10 cm^2 in area	1. Saline cleansing, silver sulphadiazine dressing applied 2. Saline cleansing, NAD applied All patients had compression bandaging	Complete healing¶ 1. 19 of 30 (63) 2. 24 of 30 (80) (n.s.) Mean reduction in ulcer area (%) 1. 1·7 2. 2·5 (n.s.)	Four of 30 patients receiving silver sulphadiazine experienced local adverse effects (erythema, pruritis) and discontinued treatment. No adverse effects were reported in the control group

Values are *mean(s.d.), †mean(s.e.m.), ‡mean (range) or §median (95 per cent confidence interval); ¶values in parentheses are percentages. RCT, randomized controlled trial; DMSO, dimethyl sulphoxide; NAD, non-adherent dressing; n.s., not significant

Three studies examined the effects of silver-based products[11–13]. A small RCT compared a silver-impregnated activated charcoal dressing with various topical agents targeted at different stages of wound healing[11]. Again, infection or colonization of the wounds was not described as a selection criterion. All wounds were debrided. There was no significant difference for complete healing at 6 weeks: OR 3·86 (95 per cent CI 0·67–22·12).

In the second study, silver sulphadiazine was compared with tripeptide–copper complex (TCC) and placebo; all dressings were secured with an elastic bandage[12]. Patients were excluded if more than 10^5 bacteria per g of ulcer tissue were detected, or if systemic or bone infection was present. Silver sulphadiazine proved to be significantly more effective than the other two preparations in terms of reducing ulcer area: mean(s.d.) reduction for silver sulphadiazine *versus* placebo was 44·0(8·2) *versus* 22·5(10·2) per cent, ES 21·50 (95 per cent CI 16·66–26·34); and for silver sulphadiazine *versus* TCC 44·0(8·2) *versus* 18·7(9·1) per cent, ES 25·30 (95 per cent CI 20·82–29·78). The values for complete healing rate were six (21 per cent) of 28 *versus* one (3 per cent) of 29 patients, OR 7·57 (95 per cent CI 0·85–67·40); and six (21 per cent) of 28 *versus* none of 29, OR 17·43 (95 per cent CI 0·93–325·15) respectively.

Finally, silver sulphadiazine was compared with saline cleansing plus non-adherent dressing in patients receiving compression therapy[13]. Neither presence of signs of clinical infection nor wound colonization was mentioned as a selection criterion. However, all ulcers were contaminated at baseline and contamination continued for most ulcers throughout the study. There were no statistically significant differences between groups for rates of complete healing or reduction in ulcer size at 12 weeks. The OR for complete healing was 0·43 (95 per cent CI 0·14–1·38).

Wounds of mixed aetiologies: systemic agents: two trials (*Table 3*)

The addition of ciprofloxacin to a topical regimen was evaluated in leg ulcers caused by both venous and arterial insufficiency[14]. The topical regimen consisted of antiseptic cleansing, debridement and application of dextranomer paste or hydrocolloid dressing. Eligible patients had a leg ulcer with isolation of an aerobic Gram-negative rod sensitive to ciprofloxacin. Patients in both groups received extra systemic antibiotics in cases of urinary tract infection, cellulitis and exacerbation of acute ulcer infection. No statistically significant differences were found for significant decrease in ulcer size (defined as 10 per cent reduction of the sum of the maximum length and width of the ulcer) or rates of complete healing after 3 months: OR 7·00 (95 per cent CI 0·71–69·16) and 3·84 (95 per cent CI 0·18–83·50) respectively. However, when significant healing and complete healing were combined, a statistically significant difference was detected in favour of ciprofloxacin: 12 (67 per cent) of 18 *versus* one (13 per cent) of eight patients, OR 14·00 (95 per cent CI 1·39–141·57).

Levamisole, normally used as a treatment for roundworm infection[33], was evaluated in patients with leg ulcers of various aetiologies[15]. Levamisole is thought to have an antibacterial action in wounds[15,34]. Clinical infection and wound colonization were not mentioned as selection criteria. At 20 weeks, all patients in the levamisole group were 'cured', compared with 76 per cent in the placebo group: OR 20·33 (95 per cent CI 1·10–375·05). It is unclear, however, if 'cure' meant complete closure of lesions.

Wounds of mixed aetiologies: topical agents: five trials (*Table 4*)

Two studies focused on evaluations of benzoyl peroxide dressings[3,16]. Different dilutions of benzoyl peroxide lotion were compared with saline when both were used to impregnate a wound dressing sponge[16]. All dressings were secured using an elastic support bandage. Each patient had at least two leg ulcers (most of venous origin) and acted as their own controls. Neither presence of signs of clinical infection nor bacterial colonization of the wound was mentioned as a selection criterion for participants. Benzoyl peroxide lotion in both 10 and 20 per cent strengths proved to be significantly more effective than saline in reducing ulcer area: mean(s.d.) remaining ulcer area at 42 days for 10 per cent solution *versus* saline 64·3(14·0) *versus* 94·7(12·7) per cent, ES 30·40 (95 per cent CI 17·84–42·96); and for 20 per cent solution *versus* saline 59·6(12·3) *versus* 93·7(15·2) per cent, ES 34·10 (95 per cent CI 21·11–47·09).

A 20 per cent benzoyl peroxide pack was compared with collagen gel in a non-randomized trial recruiting patients with leg ulcers of various aetiologies[3]. Before treatment allocation all patients received an antiseptic topical regimen until signs of infection were resolved. Patients had at least two ulcers on the same limb and acted as their own controls. The authors reported a statistically significant between-group difference in favour of the collagen gel, for reduction in wound area at 12 weeks (0·5 *versus* 15·9 per cent; $P < 0·01$) (ES not estimable).

Silver–zinc–allantoinate cream (AZAC) 1 per cent was compared with various other topical regimens in a non-randomized trial[17]. Few details of the aetiologies of the leg ulcers were reported. Infected ulcers could be included, but results were not stratified according to the presence of clinical infection at baseline. Analysis of time to healing showed that there was a statistically significant difference in favour of AZAC 1 per cent:

Table 3 Wounds of mixed aetiologies: systemic agents

Reference	Year	Methods	Participants	Interventions	Outcomes	Adverse effects and other notes
Valtonen *et al.*[14]	1989	RCT Outcomes assessed at 3 months Significant healing was defined as a 10% reduction of the maximum length and width of the ulcer	26 patients with a leg ulcer of at least 2 months' duration, with isolation of aerobic Gram-negative rod sensitive to ciprofloxacin	1. Ciprofloxacin 750 mg p.o. b.d., given for 3 months in addition to debridement and dressing 2. Treatment as above, but without ciprofloxacin All patients were given extra systemic antibiotics in cases of UTI, cellulitis and exacerbation of acute ulcer infection	Complete healing* 1. 3 of 18 (17) 2. 0 of 8 (0) (n.s.) Significant reduction in ulcer size* 1. 9 of 18 (50) 2. 1 of 8 (13) (n.s.) Complete healing and significant reduction combined* 1. 12 of18 (67) 2. 1 of 8 (13) ($P<0.05$)	Groups may not have been comparable for baseline ulcer duration. Range of duration (months) 1. 60·0–71·3 2. 29·0–35·1 The use of other antimicrobials in both groups makes results difficult to interpret Three patients in the ciprofloxacin group experienced mild nausea but none withdrew from the trial; adverse events were not reported for controls
Morias *et al.*[15]	1979	Double-blind RCT Outcomes assessed at 20 weeks	59 patients with chronic ulcers	1. Levamisole 100–250 mg p.o. for 2 consecutive days each week until cure, failure or 20 weeks 2. Identical placebo	Cure rate (%) 1. 100 2. 76 ($P<0.01$)	It is unclear whether 'cure' means the same as complete healing, as no definition is given Three of 30 patients in the levamisole group experienced gastric symptoms, but did not withdraw No adverse events were reported in the control group

*Values in parentheses are percentages. RCT, randomized controlled trial; p.o., oral route, b.d., twice daily; UTI, urinary tract infection; n.s., not significant

Table 4 Wounds of mixed aetiologies: topical agents

Reference	Year	Methods	Participants	Interventions	Outcomes	Adverse effects and other notes
Beitner[16]	1985	RCT. All patients had at least 2 leg ulcers and served as their own controls Outcomes assessed at 42 days	31 patients with at least 2 chronic leg ulcers	Group A 1. Benzoyl peroxide(20%)-moistened sponge, covered with pad, secured with support bandage 2. As above using saline instead of benzoyl peroxide Group B 3. As above using benzoyl peroxide 10% 4. Saline dressing Group C 5. As above using vehicle of benzoyl peroxide lotion 6. Saline dressing	Remaining ulcer area (%)* Group A 1. 59·6(12·3) 2. 93·7(15·2) ($P<0·05$) Group B 3. 64·3(14·0) 4. 94·7(12·7) ($P<0·01$) Group C 5. 83·6(9·6) 6. 94·4(8·0) (n.s.)	Three patients developed severe local irritation from using the 10% concentration and withdrew from the trial.
Marzin and Rouveix[3]	1982	Non-randomized trial Each patient acted as their own controls; only ulcers on the same leg were compared Outcomes assessed at 12 weeks	20 patients with 2 ulcers on the same leg	1. Collagen gel applied and covered with pad and bandage 2. Benzoyl peroxide 20% pack applied, and covered with pad and bandage Before commencing treatment all ulcers had topical regimen until no apparent infection	Wound area remaining (%) 1. 0·5 2. 15·9 ($P<0·01$)	There is no information about how the treatments were allocated to the ulcers
Margraf and Covey[17]	1977	Non-randomized trial, in which patients acted as their own controls	10 patients with chronic leg ulcers of mixed aetiologies. Infected ulcers were included	1. Left leg ulcers treated with AZAC 1% 2. Right leg ulcers treated with various topical treatments	Time to healing (days)† 1. 45(21) (19–88) 2. 104(31) (54–138) ($P<0·001$)	
Della Marchina and Renzi[18]	1997	RCT Outcomes assessed at 15 days	40 patients aged 65 years and over with DFUs or pressure ulcers	1. Antiseptic spray containing eosin 2%, chloroxylenol 0·3% 2. Alternative spray (not specified)	Complete healing (%) All wounds 1. 58 2. 30 DFUs 1. 82 2. 50 Pressure ulcers 1. 20 2. 10	Three patients in group 1 and one patient in group 2 complained of localized burning sensation Tests of statistical significance not reported
Worsley and Buchanan[19]	1991	RCT Outcomes assessed at 12 weeks	27 patients with either ulcers (aetiology not specified) or pressure ulcers	1. Hydrocolloid dressing applied, no other details given 2. Wounds covered with povidone–iodine 10% ointment, covered with NAD and gauze	Complete healing‡ 1. 4 of 12 (33) 2. 2 of 15 (13) (n.s.)	Although data are not presented per wound type, the authors state that pressure ulcers tended to show greater improvement than leg ulcers, but this difference was not statistically significant

Values are *mean(s.d.), or †mean(s.d.) (range); ‡values in parentheses are percentages. RCT, randomized control trial; AZAC, silver zinc-allantoinate cream; DFU, diabetic foot ulcer; NAD, non-adherent dressing; n.s., not significant mean(s.d.) 45(21) *versus* 104(31) days, ES 59·0 (95 per cent CI 34·12–83·88).

In a RCT recruiting patients with diabetic foot ulcers or pressure ulcers, an antiseptic spray (eosin 2 per cent and chloroxylenol 0·3 per cent) was compared with an alternative spray (constituents not specified)[18]. Details of concomitant interventions were not given (e.g. pressure relief). There were no statistically significant differences in complete healing between treatments for either diabetic foot ulcers (OR 4·5 (95 per cent CI 0·63–32·31)) or pressure ulcers (OR 2·57 (95 per cent CI 0·19–34·50)). Presence of clinical infection and/or wound colonization were not mentioned as selection criteria.

A fifth trial recruiting patients with leg ulcers (aetiologies not specified) or pressure ulcers compared a povidone–iodine dressing with a hydrocolloid dressing[19]. No details were given about whether presence of signs of clinical infection or wound colonization influenced selection of participants. At 12 weeks, there was no statistically significant difference between groups for complete healing: OR 0·31 (95 per cent CI 0·05–2·08).

Pressure ulcers: prevention: two trials (*Table 5*)

In one RCT, elderly inpatients without pressure ulcers received either a hexachlorophane-containing lotion or an inert lotion[20]. All patients had bed cradles, were turned at intervals of 2 h, and had regular washing of pressure areas with reapplication of lotion. There were no statistically significant differences between groups for mean time to develop new lesions, or for the incidence of new lesions: OR for the latter 0·87 (95 per cent CI 0·46–1·65). Initially 319 patients were recruited, but analysis was based on 167 who remained at the 3-week assessment.

In another RCT, chair-bound patients without pressure ulcers were recruited and received either a hexachlorophane-containing lotion or a cetrimide-containing lotion[21]. Existing pressure relief regimens were continued for all patients (not described). There was no statistically significant difference between groups for change in skin condition at 3 weeks: OR 1·80 (95 per cent CI 0·68–4·72).

Pressure ulcers: treatment: three trials (*Table 6*)

One study compared an oxyquinoline-containing ointment with a standard emollient[22]. There were no details of other pressure area care. For stage I lesions (erythema), there were no statistically significant differences between treatments for complete healing, improvement in lesions or time to healing. When improvement and complete healing were combined as an outcome, the authors reported the difference in healing rates as significantly higher in the oxyquinoline group; however, analysis on an intention-to-treat basis produced a non-significant difference: OR 3·47 (95 per cent CI 0·70–18·33). For stage II lesions (superficial breakdown), the oxyquinoline group achieved significantly better results for complete healing, time to healing and healed/improved lesions compared with standard emollient, but there was no statistically significant difference in the proportions of improved wounds (excludes complete healing). The OR for healed/improved lesions was 6·57 (95 per cent CI 1·30–33·34).

A povidone–iodine dressing was compared with a hydrocolloid dressing in patients with pressure ulcers staged as either II (loss of epidermal tissue) or III (presence of slough)[23]. No information was provided about general care for pressure relief. There were no statistically significant differences between groups for complete/partial healing or reduction of ulcer area at 56 days. The OR for complete/partial healing was 0·46 (95 per cent CI 0·15–1·40). No details were given about whether clinical infection/bacterial colonization of the wound influenced recruitment in either of the above studies.

A third RCT recruited elderly women with pressure ulcers contaminated with methicillin-resistant *Staphylococcus aureus* (MRSA) during the month preceding the trial[24]. However, it appeared that not all of the wounds were contaminated with MRSA at baseline. The experimental group was treated with gentian violet 0·1 per cent blended with dibutyryl adenosine 3′,5′-cyclic monophosphate as an ointment and control participants received povidone–iodine and sugar ointment. No details were given about the use of concomitant pressure relief. There was no statistically significant between-group difference for change in wound area at 14 weeks: ES 11·10 (95 per cent CI – 8·69 to 30·89). Two additional trials recruiting patients with pressure ulcers as well as other types of wounds have been reviewed under 'wounds of mixed aetiologies'[18,19].

Diabetic foot ulcers: systemic agents: two trials (*Table 7*)

A placebo-controlled trial evaluated amoxycillin combined with clavulanic acid[25]. Patients with polyneuropathy and foot lesions graded between 1A and 2A on the Wagner and Harkless scale were recruited[35]. The presence of wound surface cultures unresponsive to test medication was an exclusion criterion. There were no statistically significant differences for reduction in ulcer radius or complete healing at 20 days: ES –0·14 (95 per cent CI – 0·36 to 0·08) and OR 0·45 (95 per cent CI 0·13–1·61) respectively.

A second study compared the action of clindamycin with that of cephalexin, in patients with clinically infected wounds[26]. Systemic infection, and infection that was

Table 5 Pressure ulcers: prevention

Reference	Year	Methods	Participants	Interventions	Outcomes	Adverse effects and other notes
Green *et al.*[20]	1974	Double-blind RCT Outcomes assessed at 3 weeks	319 inpatients in elderly care wards, with Norton score of 14 or less Results are reported for 167 'completers' who remained at the 3-week assessment	1. Hexachlorophane-containing lotion applied to pressure areas 2. Inert lotion applied, similar in appearance and texture to the above All patients had bed cradles, were turned every 2 h, pressure areas washed with soap and water, and lotions re-applied at least every 6 h	Incidence of superficial pressure ulcers (%) 1. 16 2. 24 (n.s.) Time to develop superficial pressure ulcers (days) 1. 9·8 2. 8·7 (n.s.)	A large number of patients withdrew from the study, and the analysis was not conducted on an intention-to-treat basis There were two withdrawals in the hexachlorophane group, due to allergic rash, but there were no adverse events in the control group
Van der Cammen *et al.*[21]	1987	Double-blind RCT Outcomes assessed at 3 weeks and skin graded as follows: 0, normal; 1, mild persistent erythema; 2, moderate persistent erythema; 3, severe persistent erythema; 4, localized superficial blistering; 5, deep localized or extensive superficial blistering	120 chair-bound patients with Norton score 5–14	1. Buttocks/sacrum washed at least twice daily, dried, then cetrimide-containing lotion applied. Routine pressure area care was continued, but not described 2. As above except that hexachlorophane-containing lotion was used	Skin score* 1. 0·4 (0–2) 2. 0·6 (0–5) (n.s.) No. with deterioration in skin condition† 1. 8 of 60 (13) 2. 13 of 60 (22) (n.s.)	The graded skin assessment scale used is likely to produce low reliability particularly when attempting to distinguish between three different levels of persistent erythema Assessment was carried out by a single investigator, with no report of reliability checks

*Values are mean (range); †values in parentheses are percentages. RCT, randomized controlled trial; n.s., not significant

Table 6 Pressure ulcers: treatment

Reference	Year	Methods	Participants	Interventions	Outcomes	Adverse effects and other notes
Gerding and Browning[22]	1992	Double-blind RCT Outcomes assessed at 28 days	74 residents of long-term elderly care facilities, with one or more pressure ulcers graded as either stage I (erythema persisting for longer than 30 min after relief of pressure) or stage II (superficial breakdown involving the epidermis and dermis)	1. Oxyquinoline-containing ointment applied at least 3 times daily 2. A standard emollient applied at least 3 times daily No details were given about the use of pressure-relieving surfaces	Complete healing (%) Stage I 1. 58·5 2. 57·1 (n.s.) Stage II 1. 44·5 2. 21·8 ($P < 0·05$) Improved lesions (excluding complete healing) (%) Stage I 1. 31·7 2. 21·4 (n.s.) Stage II 1. 42·2 2. 34·8 (n.s.) Lesions healed or improved (%) Stage I 1. 90 2. 72 ($P = 0·05$) Stage II 1. 87 2. 57 ($P < 0·03$) Time to complete healing (days) Stage I 1. 6·2 2. 7·3 (n.s.) Stage II 1. 7·8 2· 13·0 ($P < 0·05$)	Nurses' subjective (unblinded) impression was that the oxyquinoline-containing ointment was more effective than the standard emollient (data collected by questionnaire)
Huchon[23]	1992	RCT, multicentre Outcomes assessed at 56 days	76 patients with pressure ulcers graded as either stage II (loss of epidermal tissue) or stage III (slough or slough with loss of substance)	1. Hydrocolloid dressing applied to debrided lesions. Dressings changed at least weekly 2. Debrided lesions dressed with povidone–iodine-impregnated gauze Dressings changed at least every other day No information was given about general pressure relief in either group	Improved ulcers (complete and partial healing combined) (%) 1. 84 2. 71 (n.s.) Mean rate of ulcer area reduction per week (%) 1. 10 2. 7	No adverse effects were reported for either group
Toba *et al.*[24]	1997	RCT Outcomes assessed at 14 weeks	19 women with pressure ulcers	1. Ointment containing gentian violet 0·1% and dibutyryl cAMP applied to wound 2. Povidone–iodine (concentration not stated) and sugar ointment No information was given about general pressure relief in either group	Baseline area remaining (%)* 1. 44·6(12·9) 2. 55·7(24·0) (n.s.)	No adverse effects observed in either group

*Values are mean(s.d.). RCT, randomized controlled trial; cAMP, adenosine 3′,5′-cyclic monophosphate; n.s., not significant

Table 7 Diabetic foot ulcers: systemic and topical agents

Reference	Year	Methods	Participants	Interventions	Outcomes	Adverse effects and other notes
Chantelau *et al.*[25]	1996	Double-blind RCT Outcomes assessed at 20 days	44 diabetic patients with foot lesions graded 1A to 2A, according to Wagner and Harkless classification .	1. Amoxycillin 500 mg plus clavulanic acid 125 mg p.o. t.d.s 2. Identical placebo All wounds were debrided and standard topical regimen applied including pressure relief (half-shoe, crutches and wheelchairs)	Reduction in ulcer radius (mm/day)* 1. 0·27 (0·15–0·39) 2. 0·41 (0·21–0·61) (n.s.) Complete healing (%) 1. 27 2. 45 (n.s.)	
Lipsky *et al.*[26]	1990	RCT Outcomes assessed at 2 weeks	60 male diabetic outpatients with clinically infected lesions (not immediately threatening to life or limb)	1. Clindamycin 300 mg p.o. q.d.s. for 2 weeks 2. Cephalexin 500 mg p.o. q.d.s. for 2 weeks All lesions debrided at initial evaluation	Complete healing† 1. 10 of 25 (40) 2. 9 of 27 (33) (n.s.) Improved lesions† 1. 14 of 25 (56) 2. 18 of 27 (67) (n.s.)	Three patients (one in clindamycin group) experienced gastrointestinal symptoms, but were not reported as withdrawals from the trial
Vandeputte and Gryson (unpublished)		RCT	29 patients with insulin-dependent diabetes and foot ulcers	1. Wounds cleaned with saline and 8% vinegar acid, and covered with a hydrogel dressing. Wound cavities were filled with an alginate dressing 2. Wounds treated twice daily, irrigated with chlorhexidine 0·05% solution and covered with dry gauze	Complete healing at 3 months† 1. 14 of 15 (93) 2. 7 of 14 (50) ($P<0·05$) Need for additional systemic/topical antimicrobials during the trial† 1. 1 of 15 (7) 2. 14 of 14 (100) ($P<0·0001$)	

*Values are mean (95% confidence interval); †values in parentheses are percentages. RCT, randomized controlled trial; p.o., oral route; t.d.s.; three times daily; q.d.s., four times daily; n.s., not significant immediately threatening to life or limb, were exclusion criteria. No statistically significant differences were reported between treatments for complete healing or improvement in lesions: OR 1·31 (95 per cent CI 0·43–4·00) and 0·66 (95 per cent CI 0·23–1·91) respectively.

Diabetic foot ulcers: topical agents: one trial (*Table 7*)

Chlorhexidine cleansing plus dry gauze was compared with a hydrogel dressing (J. Vandeputte and L. Gryson, unpublished report). Patients with infected ulcers were not excluded. Although use of systemic antibiotics at entry to the study was an exclusion criterion, all 14 patients in the chlorhexidine group and one of 15 in the hydrogel group received additional systemic or topical antibiotics during the trial. At 3 months, significantly more in the hydrogel group had experienced complete healing: 14 (93 per cent) of 15 *versus* seven (50 per cent) of 14, OR 0·07 (95 per cent CI 0·01–0·70). An additional trial recruiting patients with diabetic foot ulcers or pressure ulcers has been reviewed under 'wounds of mixed aetiologies'[18].

Pilonidal sinuses: systemic agents: three trials (*Table 8*)

Intravenous cefoxitin given before excision and primary suture was compared with the same procedure without antibiotics[27]. Presence of acute abscess was an exclusion criterion. There was no significant difference between groups for initial time to healing (approximately 5 weeks) or for postoperative complications. The ES for time to healing was 0·50 (95 per cent CI – 1·54 to 2·54).

In a second trial, patients with chronic discharging pilonidal sinuses were allocated to excision only, excision with suture, or excision with suture and clindamycin[28]. There were no statistically significant differences in healing rates. The ORs were 0·75 (95 per cent CI 0·15–3·65) for excision only *versus* excision with suture, 0·78 (95 per cent CI 0·16–3·77) for excision only *versus* excision with suture and clindamycin, and 1·03 (95 per cent CI 0·24–4·53) for excision with suture *versus* excision with suture and clindamycin. However, time to healing was significantly different between excision only and excision with suture (64 *versus* 14 days; $P < 0.001$), and between excision only and excision with suture and clindamycin (64 *versus* 11 days; $P < 0.001$), but the difference between excision with suture and excision with suture and clindamycin was not statistically significant. Group rates of disease recurrence were similar up to 3 years.

Oral metronidazole commencing 7–14 days after excision resulted in faster healing than the same surgery without antibiotics: mean(s.d.) time to healing 17·7(21·9) *versus* 38·5(43·6) days, ES 2·08 (95 per cent CI – 0·13 to 4·29)[29]. The presence of infection/wound colonization was not mentioned as a recruitment criterion.

Pilonidal sinuses: topical agents: three trials (*Table 9*)

One RCT assessed the effectiveness of postoperative topical gentamicin[30]. All patients underwent excision, with abscesses lanced before operation. One group had a gentamicin-impregnated collagen sponge inserted into the excised area; the other group did not receive a sponge. The rate of primary healing was significantly higher in the sponge group: 88 *versus* 35 per cent, OR 13·00 (95 per cent CI 4·16–40·67). However, it was unclear whether the higher healing rates were due to the gentamicin, the sponge or both. There was no disease recurrence at 1 year.

Two trials assessed antiseptic packs *versus* silastic foam dressings[4,31]. In one study, all patients underwent excision followed by a flavine pack for 4 days[31]. Patients then received either daily packing using chlorhexidine-soaked gauze, or silastic foam dressing remade weekly. There were no statistically significant differences between groups for time to healing or length of hospital stay. The ES for time to healing was 8·50 (95 per cent CI – 1·97 to 18·97).

In the other study, patients underwent excision of pilonidal sinuses followed by insertion of a half-strength Eusol pack[4]. The Eusol pack was removed after 48 h and patients received either continuation of the same, or silastic foam dressing, remade as required. No statistically significant differences between groups were found for length of hospital stay or time to healing (ESs not estimable). No details were given about whether clinical infection or bacterial colonization of the wound influenced selection of participants for either study.

Discussion

Current evidence

Many of the studies included in this review found no statistically significant differences between the outcomes of different treatments. Such 'negative' results can be useful findings in themselves. The need for underpowered studies to be replicated is highlighted and, where no treatment advantage has been demonstrated in studies with sufficient power, clinicians may be prompted to use other strategies, such as cost minimization, to select treatments. This review found little evidence to support the routine use of systemic antibiotics in patients with chronic wounds. However, it is important to note that consensus among experts recommends the use of systemic antibiotics in cases of acute

Table 8 Pilonidal sinuses: systemic agents

Reference	Year	Methods	Participants	Interventions	Outcomes	Adverse effects and other notes
Sondenaa *et al.*[27]	1995	RCT	51 patients with chronic pilonidal sinus disease	1. Cefoxitin 2 g i.v. given before excision and primary suture 2. Treatment as above, with no antibiotic prophylaxis	Initial time to healing (weeks)* 1. 4·0 (1·9–12·6) 2. 3·9 (1·9–11·1)	
Kronborg *et al.*[28]	1985	RCT	99 patients with chronic discharging pilonidal sinuses	1. Excision only 2. Excision with suture 3. Excision with suture and clindamycin 600 mg i.m. preop., then 150 mg p.o. q.d.s. for 4 days	Primary healing rate (%) 1. 91 2. 89 3. 88 Time to healing (days)* 1. 64 (17–157) 2. 14 (9–203) 3. 11 (7–102) ($P < 0·001$ 1 *versus* 2 and 1 *versus* 3, n.s. for 2 *versus* 3)	No adverse effects observed with clindamycin use
Marks *et al.*[29]	1985	Non-randomized trial, using alternate allocation	40 patients with pilonidal sinuses	1. Excision of pilonidal sinuses, followed by metronidazole 400 mg p.o. t.d.s. for 2 weeks, commencing 7–14 days postop. 2. Treatment as above, but without metronidazole	Time to healing (days)† 1. 17·7(21·9) 2. 38·5(43·6) ($P = 0·05$) Postop. development of wound pockets 1. 0 of 20 2. 7 of 20 ($P = 0·02$)	There were no data for baseline characteristics of participants and minimal information on selection criteria

Values are *median (range) or †mean(s.d.). RCT, randomized controlled trial; i.v., intravenous route; i.m., intramuscular route; p.o., oral route; q.d.s. four times daily; t.d.s., three times daily; n.s., not significant

Table 9 Pilonidal sinuses: topical agents

Reference	Year	Methods	Participants	Interventions	Outcomes	Adverse effects and other notes
Vogel and Lenz[30]	1992	RCT	80 patients with pilonidal sinuses	1. Excision followed by insertion of gentamicin-impregnated sponge, and application of pressure dressing 2. As above, without sponge	Primary healing (%) 1. 88 2. 35 ($P < 0·001$)	
Williams *et al.*[31]	1981	RCT, multicentre	80 patients with pilonidal sinuses	1. Silastic foam dressing, remade weekly 2. Daily packing with 0·5% chlorhexidine-soaked gauze All patients had excision, with flavine emulsion-soaked gauze packing for 4 days before group allocation	Time to healing (days)* 1. 66·2(26·1) 2. 57·7(19·6) (n.s.) Hospital stay (days)* 1. 8·5(12·3) 2. 7·3(6·2) (n.s.)	Data extracted from brief report
Walker *et al.*[4]	1991	RCT	38 patients with pilonidal sinuses	1. Silastic foam dressing, removed and washed twice daily. Sponge remade as required 2. Half-strength Eusol wick applied, changed twice daily initially, then once daily when the wound was clean All patients had excision, with initial application of half-strength Eusol wick, removed 48 h postop., before group allocation	Hospital stay (days)† 1. 12·8 (6–20) 2. 15·2 (3–27) (n.s.) Time to healing (days)† 1. 30·0 (21–39) 2. 33·0 (20–46) (n.s.)	

Values are *mean(s.d.) or †mean (range). RCT, randomized controlled trial; n.s., not significant infection associated with chronic wounds (e.g. cellulitis, lymphangitis). Considering topical agents, DMSO powder[8] and silver-based products[11–13] may be useful for leg ulcers, and oxyquinoline ointment may be beneficial for pressure ulcers[22]. A gentamicin-impregnated sponge may promote postexcision healing in patients with pilonidal sinuses[30]. All of this research, however, requires replication using well designed and rigorously conducted studies.

Research methodology and reporting issues

The research in this area is sometimes difficult to interpret owing to methodological problems of the primary studies. Future research should ensure sufficient statistical power, use of true randomization for RCTs, measures to help ensure baseline comparability, blinded outcome assessment, use of objective outcome measures, and appropriate methods for data analysis (e.g. survival analysis). Greater clarity of reporting is also recommended to help with interpretation of study findings[36].

Research content issues

Further research is required to establish the relationship between healing and colonization/infection of wounds and to clarify these definitions in terms of chronic wounds. Follow-up should include assessment of development of resistance to antimicrobial agents. Detailed reporting of concomitant physical interventions is required, as these may exert important effects on healing[37,38]. It may be useful to evaluate the effectiveness of physical interventions both relative to, and combined with, antimicrobial agents. No cost-effectiveness studies of systemic or topical antimicrobial agents were identified. The cost effectiveness of these agents needs to be established, taking into account the patterns of healing and recurrence that can occur with chronic wounds. Finally, it is important that future research includes both comparisons between antimicrobial agents and placebo, and comparisons involving combinations with other treatment strategies, since the latter are more likely to reflect clinical practice.

Wound microbiology and healing

The relationship between bacterial colonization and wound healing remains unclear[39–43]. Although it has been proposed that higher bacterial counts may be associated with failure to heal[5,40,42], some sources suggest that the presence of bacteria is unimportant[39,41]. Other findings indicate that, although the presence of any one specific bacterial group may not be detrimental to healing in leg ulcers, the presence of four or more bacterial groups may be associated with delayed healing[44].

Many evaluations of antimicrobial agents focus on microbiological outcomes, e.g. wound cultures/sensitivities, bacterial counts and eradication. Studies reporting only these types of result were excluded from this review since these intermediate or surrogate outcomes have not been shown to be accurate and reliable indicators of healing, and may be misleading[45]. Details of studies that have been excluded from this review are available on the worldwide web (http://www.hta.nhsweb.nhs.uk/).

Baseline status of wounds

Some of the variation in study results may be due to included trials having different criteria for selecting participants on the basis of whether wounds were infected or colonized at baseline. For two studies, the presence of signs of clinical infection was an inclusion criterion[26,28]; in a further two, infected wounds could be included, but results were not stratified according to the presence of baseline infection (J. Vandeputte and L. Gryson, unpublished report)[17]. In two studies, the presence of clinical infection was an exclusion criterion[5,8], and in another patients received a topical regimen designed to resolve signs of infection before treatment allocation[3]. For studies of pilonidal sinuses, the presence of discharge was an inclusion criterion in one case[28], in another the presence of acute abscesses was an exclusion criterion[27], and for a third any abscesses present at baseline had to be lanced before treatment[30].

Several other reports specified inclusion criteria in connection with the bacterial colonization (as opposed to clinical infection) of wounds at baseline. For three trials, positive bacterial wound surface culture at baseline was a participant inclusion criterion[5,14,25]. For a fourth trial, eligible patients had pressure ulcers contaminated with MRSA during the month before the trial[24]. In another study, all ulcers were contaminated at baseline but this was not specified as an inclusion criterion[13]. In another case, patients with more than 10^5 bacteria per g of ulcer tissue detected by biopsy of the lesion were excluded[12]. Several other reports did not mention presence of infection or colonization as selection criteria for participants[4,6,7,9,10,11,15,16,18,19,22,23,29,31]. However, in one of these reports, patients developing infected lesions during the trial period were withdrawn[10].

Given this variation in selection criteria across the included studies and the differences between studies in other participant characteristics, wound types, interventions and outcome measurements, it is difficult to identify a pattern of healing and efficacy of interventions in relation to presence of infection or colonization at baseline. This could only be assessed if a group of more homogenous studies was available, or if sufficient baseline data were provided to allow this to be taken into account in the analysis.

Four trials of venous leg ulcers included subgroup analyses in order to assess the impact of wound infection or bacterial colonization on healing[5,6,8,13]. Some findings indicated that ulcer infection had a significantly detrimental effect on healing[8]. For bacterial colonization, one study showed a statistically significant association between positive post-treatment wound cultures and lower healing rates[5], but other analyses suggested that bacterial contamination of ulcers did not appear to delay healing[6,13]. In an additional study of pressure ulcers, the presence of MRSA was significantly associated with a smaller reduction in wound area at 14 weeks, but these results were not presented according to treatment arm[24]. It should be noted that the numbers used for analysis in each of these studies were small and so these findings should be interpreted with caution.

Conclusions

There is little existing evidence to support the use of antimicrobial agents for chronic wound healing. Even with interventions that appear to be promising, further, more rigorous evaluation is required before use becomes routine. It is possible that physical treatments (compression bandaging, pressure relief) are more important for healing. Current evidence is insufficient to support the discontinuation of any of the agents reviewed.

Acknowledgement

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Review

Experimental models of Gram-negative sepsis

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Background: The mortality rate from sepsis has improved little over the past two decades. One reason for this has been the use of flawed or inappropriate experimental models in preclinical sepsis studies.
Methods: A literature review of animal models of sepsis was performed following a Medline search based on the following medical subject headings: disease models, endotoxin, inflammation, peritonitis and sepsis. Additional references were identified from the papers identified in the search.
Results and conclusion: Many animal models of sepsis have been described but none has proved to be superior. Extrapolation of results from endotoxicosis or bacterial infusion models should be regarded with caution. Peritonitis models should be accepted as the 'gold standard' but the use of appropriate virulent bacterial species needs to be ensured. A standardized panel of animal models for the preclinical assessment of immunomodulatory agents should be established, including at least one immunosuppressed model to simulate the immunocompromised patient with sepsis. A uniform and valid definition of sepsis applicable to both small and large animal species is required.

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Introduction

Sepsis and the systemic inflammatory response syndrome are the commonest causes of death in adult intensive care units[1,2]. Despite advances in critical care medicine, the mortality rate has not decreased significantly during the past two decades[3]. Encouraging results from animal studies of novel immunomodulatory agents engendered enthusiasm for clinical trials but the results of these have been disappointing[4]. The management of the critically ill patient with sepsis remains largely supportive[5]. A factor in the apparent failure of many clinical trials of novel immunomodulatory agents has been the use, in preclinical studies, of flawed or inappropriate animal models. That so many modifications of simple techniques exist shows that none has proved to be superior, with many failing to reproduce the cardiopulmonary and metabolic responses seen in clinical practice[6]. Concern has been expressed about the rapidity with which potential therapeutic agents have progressed from simple animal studies to clinical trials without their efficacy being confirmed in a range of models[7].

In this review, species differences in the pathophysiology of sepsis will be highlighted. The different animal models will be described, with the responses observed compared with those seen in humans. The selection of appropriate bacterial species will be discussed. With an exponentially expanding literature on sepsis this review is not intended to be exhaustive. Instead, it concentrates on representative examples of each model type. Results are placed in the context of our current knowledge of sepsis and recommendations for the conduct of preclinical studies of immunomodulatory agents are presented.

Methods

A Medline search of the literature from 1980 to 2000 was performed using the following medical subject headings and search methodology: disease models and endotoxin, or inflammation, or peritonitis, or sepsis, or sepsis syndrome. In total, 322 relevant papers were retrieved and further articles were identified from the reference lists of these papers. The review process concentrated on the design of the animal models. The influence of species selection, model design and bacterial virulence was examined against the background of the failure of clinical immunomodulatory trials.

Species selection

Small mammals have often been used in sepsis research as they are relatively inexpensive, genetically similar and available pathogen free. Survival has usually been the primary endpoint. Large mammals have often been utilized in physiological studies requiring invasive monitoring. The pig has been popular as its cardiovascular, renal and gastrointestinal anatomy and physiology are similar to those of humans[8]. The pulmonary vasculature of the sheep permits assessment of both blood and lymphatic flow, important in vascular permeability studies[9,10]. Recent changes in attitude towards the use of primates and dogs have significantly limited their use. However, the immunological similarity of primates, particularly the baboon[11], to humans makes them valuable in the assessment of cytokine responses[12].

Endotoxin sensitivity varies between species[13,14]. Furthermore, within species the response to infection can be influenced by many factors[15]. Sex, maturity, diet, oestrus state and concomitant infections are all important. Rats in proestrus have increased resistance to infection compared with those at other points of the oestrus cycle[16]. Neonatal rats have a reduced ability to survive septic challenges but resistance to infection improves rapidly during the first weeks of life[17]. Animals fed a meat diet have a reduced risk of death from a septic challenge[6] and maternal milk protects rats against peritonitis but not the effects of endotoxin[18].

Animals with either congenital or acquired defects in the immunoinflammatory cascade have made a major contribution to the understanding of the pathophysiology of sepsis. Defects may affect individual cytokines, cell lines or the whole of the immune system. Some immunodeficiencies allow sepsis to develop from endogenous or exogenous doses of organisms similar to those encountered in clinical practice[19]. Neutropenic rats are susceptible to infection with *Pseudomonas aeruginosa* with almost all animals becoming bacteraemic at the onset of fever[20]. Untreated animals rapidly die with the bacterium identified in many organs. The cytokine response profile is similar to that of humans[21]. No consensus exists regarding the most clinically relevant immunocompromised model but such models are important as sepsis is being diagnosed increasingly in immunosuppressed patients.

The development of transgenic techniques has allowed the production of animals with either deletions or overexpression of specific gene products. Vectors can delete or inactivate a specific gene in embryonic stem cells and these can be reimplanted into embryos[22]. Offspring may be crossbred to produce pups that are homozygous for the altered gene. The use of these strains has been important in defining the molecular pathways underlying the immunoinflammatory cascade. However, caution is needed in the interpretation of results as compensatory underexpression or overexpression of other gene products may occur[23].

Animal models of sepsis

Gram-negative sepsis may be induced in animals by several mechanisms[24]. Lipopolysaccharide (LPS) or bacteria can be administered parenterally, or the natural defences of the gastrointestinal tract can be breached surgically to allow endogenous bacteria to establish a focus of infection. Abscesses can be generated in soft tissues of the limbs[6]. Most models of sepsis have been developed in a haphazard fashion, influenced by local interests and resources. With the limitations of some of these models recognized, a hierarchy of species and models has begun to be identified[25].

Endotoxicosis models

LPS is a stable, relatively pure compound that can be stored in lyophilized form. An accurate dose can be measured that may be administered as a bolus or infusion. This has formed the basis for the simplest sepsis model and many endotoxicosis models have been described. Small mammals and baboons are relatively insensitive to the effects of endotoxin, whereas rabbits, sheep and chimpanzees show an enhanced response[26]. In insensitive animals, presensitization with killed organisms or D-galactosamine reduces the dose of LPS needed to produce an inflammatory response[27]. Despite the lower doses being more physiological, most endotoxicosis studies have continued to use high doses in non-sensitized animals. The duration and route of administration have varied between studies.

In rats, a high-dose bolus injection of LPS produces vasodilatation, rapid cardiovascular collapse and early death[28]. In contrast, lower doses produce a hyperdynamic response with an early increase in cardiac output[29–31]. In large mammals, a low dose of LPS produces a polyphasic response characterized by an early reduction and a late increase in cardiac output[32,33]. Prolonged low-dose infusion produces a prolonged hyperdynamic state characterized by an increased cardiac output and vasodilatation[34]. Most primate endotoxicosis models have used massive intravenous doses that resulted in rapid circulatory collapse and early death[35]. Several authors have questioned the relevance of this acute endotoxin intoxication to the more indolent course of human sepsis[6]. A low-dose infusional model has been developed in primates, more closely resembling human sepsis with subclinical coagulopathy and late multiorgan dysfunction[36].

As the care of intravenous lines in unrestrained animals is difficult, the effects of chronic LPS infusion in unanaesthetized animals have not been investigated extensively. Fish and Spitzer[37] investigated the effects of chronic LPS administration via osmotic minipumps in unanaesthetized rats. Animals developed hyperdynamic sepsis with anorexia, raised white cell counts and increased serum lactate levels. Myocardial performance was impaired[38] but coronary artery flow was maintained[39]. LPS infused into the portal vein of guinea pigs for 5 days produced metabolic features similar to those found in the rat. A correlation was noted between the clinical response and plasma endotoxin levels[40].

Endotoxicosis in humans

The development of sensitive endotoxin assays has allowed the measurement of serum LPS levels in both human volunteers and patients with sepsis. Bolus infusion of LPS in human volunteers produces a rapid increase in serum levels followed by a precipitous fall[41]. Prolonged low-dose infusion in healthy volunteers induces influenza-like symptoms with fever, tachycardia, raised white cell count and a catabolic state[42,43]. Circulating cytokine levels are an order of magnitude lower than those produced by a bolus infusion of LPS, but similar to those of patients with sepsis[44].

Detectable levels of LPS are identified in up to 75 per cent of patients with sepsis in the intensive care setting[45]. Serum endotoxin levels often remain undetectable in more indolent forms of uncomplicated sepsis[46,47] with the recorded levels being of no prognostic significance[48,49]. Very high levels are occasionally found in meningococcal septicaemia and at the start of bactericidal antibiotic therapy[50]. The highest levels recorded are usually below 2×10^{-7} g/L[51]. These are considerably lower than those measured in experimental Gram-negative sepsis when values above 10^{-5} g/L have been reported[52].

Intravenous bacterial infusion models

Several studies have investigated the response to intravenous administration of live bacteria. Many different aerobic bacterial species have been investigated; *Escherichia coli* is the most common. As with endotoxicosis models, the dose of organisms and duration of infusion have varied considerably between models. In small mammals, low doses of *E. coli* administered over several hours have been associated with minimal early physiological changes[53]. By contrast, higher doses have often produced a biphasic response with an early rise and late fall in cardiac output[54]. In large mammal studies, higher microbiological doses have invariably been used. In a baboon model, Hinshaw *et al.*[55–57] administered an LD_{100} dose of *E. coli* over 2 h, assessing death as the primary endpoint. Consistent physiological disturbances were noted with, not unsurprisingly, early death in the untreated groups. The use of fluid resuscitation has varied between studies but, in its absence, early death was often reported. Crocker *et al.*[58] developed a porcine model using both Gram-positive and Gram-negative bacteria. The haemodynamic and pulmonary changes recorded were dependent on the bacterial species used. *Staphylococcus aureus* induced minimal changes whereas both *E. coli* and *P. aeruginosa* resulted in shock and acute respiratory failure[59]. In an ovine model Booke *et al.*[60,61] infused a low dose of *P. aeruginosa* over 2 days. All animals developed a hyperdynamic cardiovascular response with vasodilatation and increased cardiac output. In a porcine model, *P. aeruginosa* infused over a week resulted in biphasic changes to the cardiac output, with late systemic hypotension and pulmonary hypertension[62].

Bacteraemia in humans

In animal models, bolus infusion of large doses of live bacteria has produced reproducible but dramatic physiological responses. These studies have often been interpreted as confirming a therapeutic intervention to be of benefit in clinical sepsis. As with acute endotoxin intoxication, the relevance of this type of model to clinical practice is unproven. Bacteria infused in high doses do not colonize and replicate *in vivo*. This is unlike any clinical situation in which organisms are released intermittently from a septic focus, in variable doses, over an extended period.

Peritonitis models

Peritonitis may be induced in animals in several ways. Bowel can be perforated allowing contamination with gastrointestinal contents, or an inoculum of faecal material or pure bacterial cultures can be instilled into the peritoneal cavity. Early models isolated segments of intact bowel and awaited the development of peritonitis[63,64]. The disadvantage of this method was that the onset of peritonitis was uncontrolled and dependent on the timing of gastrointestinal perforation. To overcome this limitation, the simple and reproducible caecal ligation and puncture model was developed and has been used widely in sepsis research[6]. The caecum is ligated distal to the ileocaecal valve and perforated using two needle punctures. The model was originally described in rats but has been extended to other species[65,66]. This technique, without fluid resuscitation, results in the rapid onset of shock[67]; with fluid resuscitation, the mortality rate may be reduced with pathophysiological responses resembling those noted in human sepsis[68].

The gastrointestinal contents of animals, particularly herbivores, vary between species[69]. Initial attempts at inducing peritonitis by intraperitoneal implantation of faeces were often disappointing, with animals appearing tolerant to their own faecal flora[70]. To overcome this, human faeces were used[71], or barium sulphate[72,73], bile salts[74] or autologous haemoglobin[75] added to the faecal material. Peritonitis induced in rats by a standard inoculum of pooled faecal material derived from several animals produced a hyperdynamic sepsis response, with a high mortality rate in unresuscitated animals[76,77].

Both the caecal ligation and puncture and faecal inoculation models deliver a variable microbiological dose and so pure bacterial culture peritonitis models have been developed. These allow both the type and dose of organism implanted to be closely controlled. Some have used single-organism cultures whereas others have used mixed cultures that more accurately mimic the gastrointestinal flora[78]. In the rat, intraperitoneal infusion of *E. coli* produces the typical metabolic and histological changes of clinical sepsis[79,80]. Similar models described in large mammals[81,82] were associated with a high mortality rate and consequently techniques were developed to administer the bacterial inoculum embedded in a fibrin clot. This reduced the mortality rate and allowed the development of chronic intraperitoneal abscesses. In a porcine model, Goldfarb *et al.*[82] implanted an *E. coli*-laden fibrin clot. Surviving animals had a period of cardiovascular impairment followed by recovery with the responses very similar to those noted in human sepsis.

To make the cultures implanted into the abdomen more closely reflect faecal contamination, yet still maintain control over the bacterial load, autoclaved faecal pellets containing both *E. coli* and *Bacillus fragilis* have been described[83]. A synergistic effect was described with all surviving animals developing localized intraperitoneal abscesses. Similar pathophysiological responses have been observed with gelatin capsules containing sterile rat faeces and *E. coli*[84]. In a canine peritonitis model induced with either *E. coli* or *S. aureus*, similar haemodynamic changes and mortality rates were seen, irrespective of the infecting organism[85]. In a porcine model, Karazian *et al.*[86] directly compared the induction of peritonitis with autologous faecal contents and an *E. coli* culture. Markedly different physiological responses were found between the two groups. Intraperitoneal inoculation of *E. coli* produced an acute illness characterized by cardiovascular collapse. Of the 70 per cent of animals that survived the initial illness all showed a rapid recovery. Intraperitoneal implantation of faeces produced a milder illness with an increased cardiac output and reduced vascular resistance. All animals survived with persistent, but less severe, physiological disturbances.

Studies that have directly compared the pathophysiological responses in endotoxicosis and peritonitis models of sepsis have shown similar mortality rates, but the kinetics and magnitude of the cytokine responses are markedly different[87]. Furthermore, responses to immunomodulatory agents have been different in the two types of model. When using intravenous endotoxicosis and peritonitis models, the cytokine and clinical responses to antitumour necrosis factor α therapy have often been divergent[88,89]. Improvements in mortality rate have been recorded in the endotoxicosis, but not peritonitis, models.

Peritonitis in humans

In humans, peritonitis is often the sequela of gastrointestinal perforation with systemic sepsis developing over several hours or days from an initially localized nidus of infection. Furthermore, the clinical course is often influenced by the early use of antibiotic therapy. The caecal ligation and puncture model is relevant to clinical abdominal sepsis in which polymicrobial sepsis often develops in the presence of devitalized tissue. An early hyperdynamic and a late hypodynamic phase have often been observed in this model, particularly if fluid resuscitation has been employed[90]. Pure bacterial cultures with either single or mixed organisms result in a reproducible sepsis response. While providing a controlled microbiological dose, single-organism cultures fail to reproduce the synergy seen between aerobic and anaerobic organisms. Aerobic Gram-negative organisms appear responsible for many of the acute physiological features of sepsis while anaerobes appear to contribute to the development of intraperitoneal abscesses[73]. Peritonitis models that have used single-animal or pooled faecal material, while less reproducible than either endotoxicosis, intravenous infusional or pure bacterial culture peritonitis models, produce a physiological response in most species similar to that found in humans. The presence of adjuvant vegetable and chemical material found in normal colonic contents also appears important. It is the qualitative differences between faecal inoculation and bacterial cultures, not quantitative differences in bacterial load, that determine the animals' pathophysiological responses. The addition of a fibrin clot to the culture, entrapping bacteria, produces a reproducible peritonitis and systemic response[91]. Peritonitis models, particularly in the pig or primates, are those that most closely represent the clinical situation encountered in humans.

Microbiological aspects of sepsis models

While detailed consideration has been given to the technical aspects of sepsis models, the choice of bacteriological inoculum has often been neglected[92]. Bacteria express a broad array of structural and soluble cell wall components that are important in the pathogenesis of infection. Bacteria with smooth LPS, acid polysaccharide capsules and those expressing K antigens are often resistant to complement-mediated bacteriolysis, and this is translated into their ability to survive *in vivo*. *E. coli* K12 lacks a polysaccharide capsule, has a rough LPS phenotype and is lysed by 10 per cent normal human serum. It survives poorly in the bloodstream in animals. In contrast, *E. coli* O18 : K1 : H7 has a smooth LPS phenotype and is not killed by 80 per cent normal human serum or neutrophils in the absence of specific antibodies[93]. The O serotype determines the ability of the bacterium to disseminate and survive outside the intestine. Serotypes O86 and O111 do not cause extra-intestinal dissemination in sepsis and they are more than a million times less virulent than O18. As a result, in non-human primates a massive bacterial dose is needed to produce any systemic response with avirulent serotypes[94]. If, as is often the case, these organisms are used in an animal model, a large inoculum is required to overcome the host defences, producing a picture of acute intoxication rather than infection. This is supported by the fact that in mice there is little difference in physiological response to a large bolus of live *E. coli* and the same dose of heated killed bacteria[95]. Few have directly compared the sepsis response to different bacterial strains in the same model. Evans *et al.*[96] compared the ability of bactericidal/permeability-increasing (BPI) protein to protect mice from death following intraperitoneal challenge with various *E. coli* strains. BPI protein prevented death in animals challenged with J5 rough strain, but not with the smooth strains O111 : B4 or O7 : K1. The bacterial growth phase is also an important factor in the surface expression of O side chain and capsular polysaccharide. Bacteria harvested in the log growth phase have a more complete LPS and a higher content of capsular polysaccharide[97]. Growth phase affects the behaviour (e.g. LD_{50}) of the strain *in vivo* and this is important in animal models using cultured organisms.

Two-hit models

Despite the observation that hypovolaemic shock may induce a state of prolonged immunosuppression, few have investigated the responses to the combined insults of hypovolaemia and sepsis that are often encountered in the critically ill patient. In a rodent model, haemorrhage reduced the inflammatory response to a subsequent intraperitoneal *E. coli* challenge[98]. In a murine model, a short period of fixed-volume haemorrhage depressed cellular immune function for up to 10 days[99]. The mortality rate associated with subsequent intra-abdominal sepsis, induced at 3 days by caecal ligation and puncture, was increased. Most of these studies were not designed to mimic a clinical scenario. Porcine models that combine fixed-volume haemorrhage with peritonitis induced by either caecotomy or direct contamination with bacterial cultures have been described recently. The pathophysiological responses observed, and the effects of resuscitation, were similar to those found in clinical sepsis[100,101].

Conclusions

The ideal animal model of sepsis should be simple and reproducible with an initiating insult via a route and of a magnitude appropriate to that seen in a clinical setting. The animals should show signs of sepsis with supporting cytokine and bacteriological results. The response should be of gradual onset from a localized source of infection and should, if possible, run for an extended course. The illness should progress from localized infection through systemic sepsis to multiple organ failure. Ideally, it should occur in an unanaesthetized animal but this is often precluded by ethical and licensing restrictions.

Animal models of endotoxicosis are simple, convenient, and induce a reproducible and physiological dose-dependent response. An overwhelming short-lived illness, leading to early death, while often producing statistically significant physiological results, does not mimic the more indolent forms of clinical sepsis. Their limited relevance makes it is unwise to progress to clinical trials based on these results alone. Many of the responses noted in bacterial infusion models are the effects of acute intoxication. Fluid-resuscitated peritonitis models are the most clinically relevant.

Owing to the heterogeneous nature of patients in the intensive care unit and the cost of large-scale clinical trials, animal studies will continue to play an important role in the development of new immunomodulatory agents. If reliable information is to be obtained from these studies, a careful selection of species, models and bacteria will be required. A standardized panel of models should be established. Simple endotoxicosis and bacterial infusion models would allow ‘screening’ of potential therapeutic agents. This should be followed by a number of studies in small mammal peritonitis models, including at least one immunosuppressed model simulating the immunocompromised patient with sepsis. Specific studies should be considered in non-human primates before clinical trials.

Until 1992, a consistent definition of sepsis or systemic inflammatory response syndrome in humans did not exist[102]. As many of the above models were described before this time, it is inevitable that many differing physiological variables were measured and many different definitions of sepsis applied. There is a need for a uniform and valid definition of sepsis, applicable to both small and large animal models, that will allow comparison of different studies. In clinical practice many different organ scoring systems have been developed to monitor sepsis-induced multiple organ dysfunction syndrome[103]. Similar simple scoring systems, validated in animals, would allow objective assessment of both the sepsis response and the effects of resuscitation.

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Review

Somatostatin receptor imaging, therapy and new strategies in patients with neuroendocrine tumours

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Background: Somatostatin receptors have been found on a variety of neuroendocrine tumours, such as carcinoids and paragangliomas, as well as on most pancreatic endocrine and breast tumours. Somatostatin receptor scintigraphy with a radionuclide-labelled somatostatin analogue, [^{111}Indium-diethylenetriaminopenta-acetic acid]octreotide, is a sensitive and specific technique for visualizing *in vivo* the presence of somatostatin receptors on various tumours.

Methods: Material was identified from previous review articles, references cited in original papers and a Medline search of the literature. Additional material was obtained from recently published abstracts of meetings.

Results and conclusion: Somatostatin receptor imaging of neuroendocrine tumours is essential in the diagnostic evaluation of most of these tumours. The expression of somatostatin receptors *in vivo* not only predicts the outcome of somatostatin analogue treatment but also opens the possibility of new therapeutic strategies. Because better information about spread of the disease can be obtained, more justifiable options for therapy can be proposed.

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Somatostatin receptor expression

Somatostatin (SS) is a small regulatory peptide; it was isolated in the ovine hypothalamic gland in 1973 as a growth hormone (GH) release inhibiting factor[1]. SS is widely distributed in the human body and is found not only in the hypothalamus but also in various parts of the gastrointestinal tract, indicating that inhibition of GH is not its only function[2]. Apart from its function as a neurotransmitter in the central nervous system, it also has inhibitory effects on the secretion of hormones by the pancreatic islets (insulin, glucagon) and on exocrine pancreatic function. SS also inhibits normal gastrin production, and consequently gastric acid and pepsin production[3]. A number of observations have suggested an antiproliferative effect of SS and its stable analogues[4–6].

Critical to all these actions is the expression of SS receptors (SS-Rs) on the cell membrane. These SS-Rs subserve two functions, first to recognize the ligand and bind it with high affinity and specificity, and second to generate a transmembrane signal that evokes a biological response. Large numbers of SS-Rs are found on most tumours with amine precursor uptake and decarboxylation characteristics and neuroendocrine properties, such as carcinoids, paragangliomas, phaeochromocytomas, medullary thyroid cancers and endocrine pancreatic tumours. In addition, large numbers of binding sites with high affinity for SS are also found on breast and brain tumours, as well as on various cells of the immune system[7–10]. At least five different human SS-R subtypes have been cloned[11]. All subtypes bind SS with high affinity, while their affinities for the SS analogue octreotide differ considerably. Octreotide binds with high affinity to SS-R subtype 2 (sst_2) and sst_5, to a lesser degree sst_3, while no binding to sst_1 and sst_4 occurs. Other SS analogues that are in clinical use, such as BIM23014 (lanreotide) and RC-160 (vapreotide), as well as the hexapeptide MK678, bind to three of the five SS-R subtypes, also displaying high affinity for sst_2 and sst_5 and moderate affinity for sst_3[12].

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SS-R messenger RNA subtypes are widely expressed in neuroendocrine tumours, but their distribution does not necessarily correlate with SS-R subtype expression. Furthermore SS-R subtypes show a differential subcellular localization in human SS-R-positive tumours[13]. The majority of human endocrine pancreatic tumours, such as gastrinomas, glucagonomas, vipomas and 'non-functioning' islet cell tumours, express sst_2. *In vitro* studies have shown that 72 per cent of insulinomas express SS-Rs. However, these are mainly sst_3 receptors, which have low affinity for octreotide[14,15]. Although SS-Rs have been demonstrated on exocrine pancreatic cells in experimental animals (mainly on acinar cells), neither SS-Rs nor neuroendocrine properties could be confirmed on human exocrine pancreatic adenocarcinomas[16]. Carcinoids, paragangliomas, phaeochromocytomas and medullary thyroid cancers all have a mixed distribution of the SS-R subtypes, but sst_2 is expressed most frequently. The presence of different combinations of SS-R subtypes may explain the variable clinical response to SS analogues and the difference in successful localization by SS-R scintigraphy (SRS) of these tumours. Metastases of SS-R-positive tumours initially express the same SS-R subtype as the primary, but loss of these receptors has been described after dedifferentiation of tumour cells or after chemotherapy[17]. In addition, SS-R overexpression has been identified in peritumoral veins of primary neoplasms and in veins surrounding lymph node, bone and lung metastases[18].

Somatostatin receptor scintigraphy

The optimal management of patients with neuroendocrine tumours requires accurate imaging and staging. For the visualization of SS-R-positive tumours, SRS with [^{111}Indium-diethylenetriaminopenta-acetic acid (^{111}In-DTPA^0)]octreotide (Octreoscan, Mallinckrodt Medical BV, Petten, The Netherlands) has been used for more than 10 years[19,20]. The efficacy of SRS using this agent in patients with histologically or biochemically proven endocrine pancreatic tumours or carcinoids was evaluated in a European multicentre trial[20]. The highest success rates were observed with glucagonomas (100 per cent), vipomas (88 per cent), gastrinomas (73 per cent), 'non-functioning' islet cell tumours (82 per cent) and carcinoids (87 per cent). Insulinomas were detected in only 46 per cent of cases owing to the low incidence of sst_2 on insulinoma cells. The low sensitivity in this study found for some tumours may be related to important differences in scanning procedures, such as the amount of radioligand administered, the duration of the acquisition and the use of single photon emission computed tomography (SPECT)[21]. With SPECT, in which a rotating camera is used, image reconstructions can be made similar to those of spiral computed tomography (CT). This technique gives additional visual information, especially on tumours in the liver and upper abdomen[22]. Twenty-five per cent more liver metastases are detected with SPECT than with planar SRS.

In a prospective study comparing the sensitivity of SRS with that of CT, magnetic resonance imaging (MRI), ultrasonography and selective angiography in the detection of primary and metastatic gastrinomas, SRS altered clinical management in 47 per cent of instances and had a superior sensitivity and specificity[23] (*Table 1*). Cadiot *et al.*[24] compared the results of SRS with those of conventional imaging techniques, including endoscopic ultrasonography, and with surgical findings in 21 consecutive patients with Zollinger–Ellison syndrome. SRS added complementary information to other imaging techniques, including endoscopic ultrasonography, and improved the preoperative detection of extrapancreatic gastrinomas. By combining SRS with endoscopic ultrasonography they were able to detect 90 per cent of the tumours in the upper duodenopancreatic area. SRS identified metastatic disease in 20–30 per cent of patients after all other imaging techniques had failed[22]. In another study of 160 patients with biologically and/or histologically proven gastroenteropancreatic tumours, including pancreatic islet cell tumours, SRS changed the surgical therapeutic strategy in 40 patients (*Table 2*)[25]. Unsuspected liver tumours were discovered

Table 1 Imaging methods for the detection of liver metastases and extrahepatic metastases in patients with Zollinger–Ellison syndrome

	Positive result (%)	
Procedure	Extrahepatic tumour ($n = 80$)	Liver metastases ($n = 24$)
Ultrasonography	9	46
CT	31	42
MRI	30	71
Angiography	28	62
CIM	48	83
SRS	58	92
SRS + CIM	68	96
SRS only*	20	12
CIM only†	10	4

Results are expressed as the percentage of the 24 patients with proven liver metastases and of the 80 patients with extrahepatic disease. Conventional imaging methods (CIM) included ultrasonography, computed tomography (CT), magnetic resonance imaging (MRI) and angiography. *Tumour detected only with somatostatin receptor scintigraphy (SRS); †tumour detected only with CIM. From reference 23 only by SRS in seven patients, contralateral liver tumours before hepatectomy in two, and extrahepatic disease in 31. In 48 patients with histologically proven hepatic metastases of neuroendocrine tumours, including carcinoids, the present authors compared conventional imaging methods (CIM) with SRS for the detection of extrahepatic disease. CIM consisted of ultrasonography, CT of the thorax and abdomen and, when indicated, bone scanning. All patients were scanned according to a multiple spot views protocol. A minimal dose of 200 MBq ^{111}In and at least 10 μg of peptide was administered as an intravenous bolus, and acquisition was performed 24 and 48 h thereafter. Acquisition time was 15 min and abdominal SPECT was used systematically with a triple head camera. SRS alone demonstrated 114 extrahepatic lesions in 37 patients, whereas CIM visualized only 50 extrahepatic lesions in 22 patients (*Table 3*).

In patients with paragangliomas, SRS provides optimal information on other potential tumour sites in the body; paragangliomas are often multicentric and asymptomatic[26]. For imaging of adrenal phaeochromocytomas, scintigraphy with radiolabelled metaiodobenzylguanidine (MIBG) is more accurate than SRS, since the majority of benign phaeochromocytomas are localized in the adrenal, a localization that is difficult to distinguish from the kidney with SRS. Furthermore, the majority of these benign lesions do not express SS-Rs. Recently, in a large European multicentre study, the sensitivity of [^{131}I]MIBG in detecting benign phaeochromocytomas was shown to be 81 per cent[27], while the detection rate with SRS is only around 25 per cent. The majority of malignant tumours (80 per cent), in contrast, express appropriate receptors and so SRS could be used to characterize adrenal masses suspected to be malignant[28]. SRS has a limited role in the management of medullary thyroid cancer. It might be useful for the detection of residual and recurrent disease after total thyroidectomy, or in patients who present primarily with metastatic disease in order to study the feasibility of performing peptide receptor radionuclide therapy[29,30]. To optimize the detection of metastases in patients with recurrent medullary cancer, the combination of SRS and ^{99m}Tc-radiolabelled dimercaptosuccinic acid (^{99}Tc-DMSA) should be considered, as together they have a sensitivity of 84 per cent for the diagnosis of medullary cancer. SRS alone has a sensitivity of 29 per cent and ^{99}Tc-DMSA alone has a sensitivity of 69 per cent[31].

Finally, the difference in SS-R expression between islet cell tumours, especially 'non-functioning' tumours and pancreatic duct cancers, offers the possibility of differentiating between these tumours before surgery[32]. This is important, as palliative surgery in patients with islet cell tumours is of value not only in relieving clinical symptoms but also in decreasing tumour burden, which might enhance the effect of medical treatment and result in improved clinical status and longer survival.

Table 2 Clinical impact of somatostatin receptor scintigraphy in 160 patients with gastroenteropancreatic tumours

		SRS classification		
Initial classification	Total no.	I	II	III
I No metastases	90	65	7	18
II Liver metastases only	59		46	13
III Extrahepatic metastases	11			11

SRS, somatostatin receptor scintigraphy. From reference 25

Table 3 Detection of extrahepatic metastases: somatostatin receptor scintigraphy *versus* conventional imaging methods

	CIM negative	CIM positive	Total
SRS negative	11		11
SRS positive	15	22	37

Imaging was carried out in 48 patients with histologically proven hepatic metastases of neuroendocrine tumours. The table shows number of patients with extrahepatic disease detected by somatostatin receptor scintigraphy (SRS) with [^{111}Indium-diethylenetriaminopenta-acetic acid]octreotide or by conventional imaging methods (CIM) consisting of computed tomography, ultrasonography and bone scanning. In 15 patients extrahepatic metastases were detected with SRS only. Eleven patients were found to have no extrahepatic disease

Clinical use of somatostatin analogues

Most endocrine pancreatic tumours, with the exception of insulinomas, have a malignant potential and have already metastasized at the time of diagnosis. These tumours are in general slow growing and most of the clinical distress is related to the hypersecretion of hormones, which often incapacitates the patient and causes long and repeated periods of admission to hospital. The clinical use of the SS analogue octreotide in this type of patient is of considerable help in controlling symptomatology. Debilitating diarrhoea, dehydration and hypokalaemia (vipoma) and necrolytic skin lesions (glucagonoma) can be well controlled during continuing treatment with octreotide. There is no doubt that octreotide therapy is of great benefit for most of these patients and improves their quality of life dramatically[33]. In selected patients with peptic ulceration and hyperplasia of fundic argyrophil cells (gastrinoma) or life-threatening attacks of hypoglycaemia (metastatic insulinoma) octreotide may also be of therapeutic benefit[34,35].

Clinical studies in those with hormone-producing islet cell tumours have shown a close parallel between the presence of SS-Rs on the tumours and the *in vivo* and *in vitro* suppressive effects of octreotide on hormone release[36]. This indicates that SRS can predict a possible suppressive effect of octreotide on hormonal hypersecretion by endocrine pancreatic tumours. Although octreotide is able to inhibit gastrin release in Zollinger–Ellison syndrome, proton pump inhibitors are currently the first choice as over 80 per cent of patients are controlled by omeprazole, lansoprazole or pantoprazole. A major problem in the treatment of islet cell tumours and carcinoids with octreotide is that the inhibition of secretion of tumour-related hormones is transient. Most patients finally become insensitive to octreotide treatment, possibly by down-regulation of SS-R expression or outgrowth of SS-R-negative clones[6,37,38]. The beneficial effects on clinical symptomatology in those with metastatic endocrine pancreatic tumour and carcinoids is highly variable. Increasing the dose of octreotide or intermittent administration reverses these problems in most cases. The median duration of improvement of diarrhoea and flushing attacks by octreotide in patients with metastatic carcinoid disease is over 12 months[5].

In some patients with metastatic medullary thyroid cancer, continuous treatment with very high doses of octreotide may bring temporary relief. Long-term therapy with SS analogues for catecholamine-secreting (malignant) paragangliomas and phaeochromocytomas has not shown clinical benefit[39].

The role of octreotide in the treatment of pancreatitis is controversial. A meta-analysis of six studies showed a significant decrease in mortality rate in patients with mild or severe disease (6 per cent octreotide *versus* 14 per cent placebo)[40]. However Uhl *et al.*[41] did not find a significant difference in mortality rate or complications in a large series of 302 patients with acute pancreatitis. The decrease in digestive enzyme secretion and the increase in intestinal water and electrolyte absorption caused by octreotide can be beneficial in the treatment of pancreatic and enterocutaneous fistula[42,43]. In elective surgery for pancreatic carcinoma or chronic pancreatitis, perioperative administration of octreotide has demonstrated its value. In a study of 246 patients who underwent pancreatic surgery, octreotide treatment reduced the incidence of death and complications compared with placebo (3 and 32 *versus* 6 and 55 per cent respectively)[44]. Finally, SS and octreotide infusion reduce portal pressure by decreasing splanchnic blood flow. Although the exact mechanisms of acute haemodynamic changes in patients with portal hypertension are unknown, there is a rationale for the use of SS and its derivatives in the control of acute oesophageal variceal bleeding[45]. Early infusion of SS in cirrhotic patients with acute variceal haemorrhage decreases treatment failure and mortality rates, and reduces active bleeding after sclerotherapy, thereby improving the efficacy of sclerotherapy for acute variceal bleeding episodes[46].

Oncological applications of somatostatin analogues

The observation that SS inhibits the release of various peptide hormones has stimulated interest in its use as an antiproliferative agent. In preclinical studies SS analogues have inhibited the growth of a wide variety of SS-R-positive as well as SS-R-negative tumours *in vivo* and *in vitro*[4,6,47]. An indirect tumour growth inhibition may be achieved via the inhibition of circulating tumour growth promoting hormones (GH, insulin-like growth factor 1, insulin) and inhibition of circulating, paracrine- and/or autocrine-secreted stimulatory growth factors. SS and its analogues can also modulate the activity of immune cells[48] and potentially influence tumour blood supply; Reubi *et al.*[49] demonstrated a high density of SS-Rs on veins in the peritumoral zone of several types of malignant tumour. A potentiation of the antiproliferative effect of octreotide has also been suggested[50], but no beneficial effect of octreotide combined with tamoxifen was found in patients with metastatic breast cancer[51]. Critical to the direct antiproliferative effects of SS analogues is the presence of SS-Rs[52]. Both adenosine 3′,5′-cyclic monophosphate-dependent and -independent effector mechanisms have been suggested[53–56], while stimulation of phosphotyrosine phosphatase activity may play an important role in the inhibition of growth factor-stimulated cell growth[57,58].

The results of clinical trials in patients with gastroenteropancreatic tumours, using SS analogues alone or in combination with interferon-α, are rather disappointing, with a biochemical response in 77 per cent of patients with a median duration of 15 months but without any reduction in tumour size[59,60]. Clinically, octreotide has been the most commonly used SS analogue, yielding biochemical response rates of between 30 and 70 per cent but objective tumour shrinkage in less than 10–15 per cent of patients[5,61–64]. Treatment with standard doses of the SS analogue lanreotide does not appear to be any better than that with standard doses of octreotide. However, tumour biopsies before and during treatment with high doses of lanreotide have indicated apoptosis in responding patients[65,66]. Side-effects of long-term administration of SS analogues are rare. The most relevant side-effect is the development of gallstones, which is believed to derive from the inhibition of gallbladder emptying due to the inhibition of cholecystokinin release[67]. The incidence of developing cholelithiasis and/or gallbladder sludge is reported to be between 30 and 60 per cent, and seems to be dose dependent[68,69]. Patients require cholecystectomy for either symptomatic disease or acute cholecystitis in 15 per cent of cases; prophylactic cholecystectomy is not indicated, unless it is performed during elective cytoreductive surgery[69]. Octreotide might also influence blood sugar levels in patients with diabetes mellitus.

Radionuclide therapy: preclinical

A new and fascinating application is the use of radiolabelled octreotide and other peptides for peptide receptor radionuclide therapy, henceforth referred to as radionuclide therapy. After systemic injection of [^{111}In-DTPA^0]octreotide, the radioligand is internalized and transported into the lysosomes by an SS-R-specific and temperature-dependent process starting with endocytosis[70,71]. The radionuclide as metabolite ^{111}In-DTPA-D-Phe is not capable of passing the lysosomal membrane, resulting in a biological half-life in human tumour tissue of over 700 h[72,73]. ^{111}In not only emits γ rays, which can be visualized during scintigraphy, it also emits internal conversion and Auger electrons which have a short to medium tissue penetration (0·02–10 and 200–550 µm respectively). Therefore, an effect on tumour cell proliferation might be expected, as the radiotoxicity of the radionuclide is very high if the DNA of the cell is within the particle range[74,75].

Three experimental animal studies have demonstrated tumour growth inhibition of solid subcutaneous tumours by radiolabelled SS analogues in animal models[76–78]. The authors investigated the antiproliferative effect of [^{111}In-DTPA^0]octreotide on SS-R-positive, CA20948 pancreatic tumour cells in a rat liver metastases model with intraportal tumour cell injections[79]. Treatment with 370 MBq (0·5 µg) [^{111}In-DTPA^0]octreotide was given on day 1 and/or day 8. After 21 days the number of tumour colonies was significantly decreased by all treatment regimens compared with that in control animals; treatments on day 1 and 8 proved to be superior to single treatment on either day 1 or day 8 (*Table 4*). In repeated experiments, most treated animals showed no or few tumour colonies, an outcome that was not observed earlier in experiments using high doses of non-radiolabelled octreotide[52]. This tumour growth inhibition was predominantly due to the specific binding of [^{111}In-DTPA^0]octreotide to the SS-R and not to a systemic or a secondary mechanism since pretreatment with 1 mg octreotide before radionuclide treatment, resulting in saturation of the SS-Rs, almost completely abolished the effect of [^{111}In-DTPA^0]octreotide on SS-R-positive cells. Furthermore, radionuclide therapy had no effect on the growth of SS-R-negative CC531 tumours in the same experiment[79]. During the experiments there was no sign of toxicity in the animals and there was no histological damage to the kidney, which is the first organ to be at risk[80]. It was demonstrated earlier that liver regeneration after 70 per cent partial hepatectomy in this model accelerates tumour growth dramatically[81]. Radionuclide therapy was found completely to annihilate this tumour growth stimulation of SS-R-positive tumour cells rendering only a few tumour colonies. In an experiment without tumour [^{111}In-DTPA^0]octreotide did not influence liver regeneration or function, suggesting that radionuclide therapy might also be a safe option for adjuvant treatment after liver resection or arterial embolization. Although treatment with ^{111}In-labelled SS analogues may be a promising option for patients with locally irresectable or disseminated gastroenteropancreatic tumours, more experiments in more advanced stages of tumour development, with different doses of [^{111}In-DTPA^0]octreotide and with other radioligands, are necessary.

Table 4 Effect of radionuclide therapy with [^{111}Indium-diethylenetriaminopenta-acetic acid]octreotide on somatostatin receptor-positive liver metastases

	No. of metastases			
Treatment	0	1–20	21–100	>100
Controls				6
Therapy on day 1		4	1	
Therapy on day 8		3	2	
Therapy on days 1 and 8	3	2		

Values are number of animals with given range of metastases (5–6 animals per group), 21 days after direct injection of somatostatin receptor-positive CA20948 tumour cells into the portal vein. Peptide receptor radionuclide therapy with 370 MBq (0·5 µg) [^{111}Indium-diethylenetriaminopenta-acetic acid (^{111}In-DTPA^0)]octreotide was given on day 1 or 8 or on days 1 and 8. The effect of all treatment schedules was significantly different ($P<0{\cdot}01$) from the effect of 0·5 µg 'cold' [^{111}In-DTPA^0]octreotide on day 1 and 8 (controls). Treatment on day 1 and 8 was significantly different from treatment on day 8 alone ($P<0{\cdot}05$). No significant difference was found between the effect of treatment on day 8 or day 1. From reference 79

Another approach in treating tumours expressing SS-R is to substitute the radionuclide with a chemotherapeutic agent. Schally's group has developed a cytotoxic SS analogue containing 2 pyrrolinodoxorubicin (AN-201), which is 500–1000 times more potent *in vitro* than its parent compound[82,83]. By linking AN-201 to RC-121, an SS analogue, a new cytotoxic SS analogue (AN-238) was obtained. Various *in vitro* experiments with AN-238 on human and rat SS-R-positive tumours have shown effective inhibition of tumour growth[84,85].

Radionuclide therapy: clinical

A phase 1 study on the side-effects and antiproliferative effect of high, multiple radiotherapeutic doses of [^{111}In-DTPA^0]octreotide started in 1995[86]. Thirty end-stage patients with mainly neuroendocrine tumours were included. After scoring tumour radioactivity uptake using scintigrams obtained 24 h after the injection of a diagnostic dose (220 MBq) of [^{111}In-DTPA^0]octreotide, treatment was initiated. All patients received doses of 6–7 GBq ^{111}In incorporated in 40–50 μg [^{111}In-DTPA^0]octreotide with intervals of at least 2 weeks between administrations. A total of eight injections was aimed at, with a possible extension to 12–14 administrations. Twenty-one patients received a total cumulative dose of at least 20 GBq [^{111}In-DTPA^0]octreotide with a maximum of 75 GBq. Of the nine patients treated with a total dose lower than 20 GBq, seven had to stop prematurely because of disease progression despite treatment and two had not finished the first course of four administrations at the time of the interim analysis. With a maximum follow-up of 26 months no major clinical side-effects were observed except for a transient decline in platelet and white blood cell count. Kidney function was influenced only slightly and not to a clinically relevant degree. Although in some patients temporary because of end-stage disease, impressive effects on clinical condition and hormone or tumour marker production were observed after the administration of high doses of [^{111}In-DTPA^0]octreotide. Before the start of treatment all 21 patients had progressive disease. In eight patients treatment resulted in stable disease and in six tumour shrinkage was monitored by CT or MRI; in these patients beneficial effects occurred on hormone production and symptoms. There was a tendency towards a better result of radionuclide therapy in those whose tumours had a higher accumulation of the radioligand on the scoring scintigram. *Table 5* summarizes recent results and characteristics of patients treated with radionuclide therapy with a minimum dose of 20 GBq [^{111}In-DTPA^0]octreotide[86–91].

Table 5 Results and characteristics of patients treated with radionuclide therapy with a minimal dose of 20 GBq [^{111}In-radiolabelled diethylenetriaminopenta-acetic acid^0]octreotide

		Tumour size		
Tumour type	Total no.	Reduction	Stable	Progression
Carcinoid	13	3	8	2
Neuroendocrine tumour	6	2	2	2
Gastrinoma	1		1	
Vipoma	1	1		
Glucagonoma	1	1		
Medullary thyroid cancer	3		1	2
Papillary thyroid cancer	1		1	
Glomus tumour	2	1	1	
Phaeochromocytoma	2		1	1
Astrocytoma	1		1	
Inflammatory breast cancer	1		1	
Total	32	8	17	7

All phase 1 studies. Data are from references 86–89

For radiotherapeutic applications, other radionuclides such as ^{90}Y and ^{177}Lu have also been proposed for coupling to SS analogues. ^{90}Y, with a half-life of 2·7 days, is a pure high β emitter with a tissue range up to 1 cm and ^{177}Lu, with a half-life of 6·7 days, emits γ radiation (suitable for visualization) and intermediate β particles with approximately 1 mm tissue penetration. For tumours with a heterogeneous distribution of SS-Rs, ^{90}Y- and ^{177}Lu-labelled SS analogues might have extra beneficial characteristics because of an effect known as 'cross-fire'. A tumour cell lacking an SS-R might be hit by an electron coming from a neighbouring cell that has internalized the radioligand. This mechanism may lead to a high and more homogeneous radiation dose in larger parts of the tumour. Studies with these radiolabelled SS analogues are ongoing.

Transfection of somatostatin receptors

New developments in molecular biology have made it possible to transfect SS-R-negative tumour cells with an SS-R gene. Susini's group has developed an approach using sst$_2$ gene transfer for the treatment of pancreatic cancer[92]. By inducing the SS-R on the tumour cells, antitumour effects were obtained which might be attributed to several mechanisms. First, an autocrine negative feedback loop in which transfected tumour cells start to produce SS, which binds in an autocrine manner to the induced SS-R, may provide an inhibitory effect on tumour cell growth. Second, binding of SS to sst$_2$ may upregulate *p27*, a tumour suppressor gene, which leads to cell cycle arrest in the G0–G1 phase, and subsequently causes apoptosis. Local and distant bystander effects have also been noted[93]. The local bystander effect might be attributed in part to apoptosis. When type sst$_2$-positive cells undergo apoptosis, these cells release apoptotic vesicles and enzymes, which in turn might kill neighbouring cells. The distant bystander effect may be explained by a paracrine effect. SS can upregulate the expression of sst$_1$ on parental tumour cells, thereby rendering them sensitive to the antiproliferative effect of SS. All the abovementioned mechanisms may contribute to successful treatment of certain types of cancers with gene therapy.

Another reason why transfection of tumour cells with an SS-R gene may be beneficial involves radionuclide therapy[79]. By inducing the SS-R on SS-R-negative tumours, treatment with radionuclides should be possible. Moreover, transfection of SS-R-positive tumours with an SS-R gene can increase the homogeneity of distribution of tumour cells expressing the SS-R and so increase the efficacy of therapy; the present authors are currently investigating this strategy. By using a plasmid with complementary DNA of the SS-R and G418 genes, which provides protection against the cytotoxic drug geneticin, 10 per cent of SS-R-negative CC531 cells were cotransfected *in vitro*. Thereafter geneticin was added to the cells, such that non-transfected CC531 cells were eliminated, resulting in a tumour cell culture of 100 per cent transfected CC531 cells. To test expression and functional capacity of the transfected receptor, binding and internalization studies were performed. A mean of 12 000 receptors per cell were expressed on the cell membrane, while internalization studies showed a high uptake of radioactive octreotide in transfected cells (66 000 *versus* 1200 c.p.m. in controls; $P < 0{\cdot}01$). This effect could be blocked by 'cold' octreotide, indicating a receptor-specific transport mechanism. To evaluate growth and receptor status *in vivo*, *in vitro* transfected CC531 cells and (normal) CC531 cells were tested in a liver metastasis model by injection into the portal vein of WAG/Rij rats. Transfected CC531 cells had the same growth velocity as (normal) CC531 cells and had a high expression of SS-Rs. In future experiments, radionuclide therapy using β-emitting radionuclides, including ^{90}Y and ^{177}Lu, will be tested on these *in vitro* transfected CC531 cells. Transfecting tumour cells with SS-Rs in combination with radionuclide therapy is a new modality in the treatment of cancer; however, it is experimental and its full potential remains to be elucidated in the near future.

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Randomized clinical trial of diathermy *versus* scalpel incision in elective midline laparotomy

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Background: Electrocautery is used increasingly for tissue dissection, although fears of excessive scarring and poor wound healing have curtailed its widespread use for skin incision. This study compared electrosurgical incision with traditional scalpel incision.

Methods: One hundred patients requiring elective midline laparotomy were randomized prospectively to either scalpel or diathermy incision. Parameters measured included incision time, wound size, wound blood loss, total intraoperative blood loss and postoperative wound pain. All wound complications were recorded.

Results: The two groups did not differ significantly in relation to patient or wound characteristics. Laparotomy incisions using diathermy were significantly quicker than scalpel incisions (mean(s.e.m.) 6·1(0·4) *versus* 7·5(0·5) s/cm^2; $P < 0{\cdot}04$). There was significantly less blood loss in the diathermy group compared with the scalpel group (0·8(0·1) *versus* 1·7(0·3) ml/cm^2; $P = 0{\cdot}002$). Postoperative pain scores were significantly lower in the diathermy group for the first 48 h after operation ($P < 0{\cdot}05$). Morphine requirements were also significantly lower over the first 5 postoperative days in the diathermy incision group ($P < 0{\cdot}04$). There was no difference between groups in wound complications before discharge and at the 1-month follow-up.

Conclusion: Electrosurgical midline incision in elective surgery has significant advantages over scalpel use on the basis of incision time, blood loss, early postoperative pain and analgesia requirements.

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Introduction

While electrosurgical instruments are used increasingly for tissue dissection, concerns about excessive scarring and poor wound healing have curtailed the widespread use of electrosurgery for skin incision. The use of an electrode delivering a pure sinusoidal current, however, allows tissue cleavage without damage to surrounding areas. Electrosurgical incision of this type is not a true cutting incision[1]. This method heats cells within the tissue so rapidly that they vaporize, leaving a cavity within the cell matrix. The heat created dissipates as steam rather than being transmitted into adjacent tissues. As the electrode is moved forward, new cells are contacted and vaporized with the creation of an incision. This may explain the absence of tissue charring and the subsequent healing of tissues with minimal scarring[1].

Previous studies have compared electrosurgical and scalpel incisions individually in terms of wound infection, wound-related pain and blood loss[1–3]. No single study to date has looked at these parameters in combination, and there has been no work focusing on these parameters in elective midline laparotomy exclusively. This study compared electrosurgical and scalpel incision of all abdominal layers in terms of incision time, wound-related blood loss, postoperative wound-related pain and wound complications.

Patients and methods

One hundred consecutive patients scheduled for elective abdominal surgery via a midline laparotomy were studied. Exclusion criteria were diabetes, current warfarin use and previous midline laparotomy. Aspirin use was permitted during the study. All patients were given 20 mg enoxaparin subcutaneously as prophylaxis for deep venous thrombosis on the morning of surgery and daily while immobilized after operation. Patients were randomized prospectively into scalpel or diathermy incision groups. Randomization was by sealed envelope upon the patient's arrival in the operating suite. All participating patients provided informed written consent. The trial was approved by the local ethics committee and was performed in line with the Declaration of Helsinki.

All laparotomies were midline and all were classified as clean or clean–contaminated according to the adaptation of the classification of the American College of Surgeons committee on control of surgical infections[4]. All layers were incised with either scalpel or diathermy, and diathermy was used for haemostasis in both groups. Diathermy incisions were created using a standard diathermy pen electrode, set on cutting mode and delivering a 500-kHz sinusoidal current. Upon completion of the incision, impervious wound-edge protectors (3M, Dun Laoire, Dublin, Ireland) were inserted in all cases[5].

All patients received intravenous antibiotic prophylaxis: amoxycillin and clavulanic acid 1·2 g, and metronidazole 500 mg at induction and for two postoperative doses. Patients allergic to penicillin were given 1 g erythromycin with metronidazole. Wound closure was achieved by the technique of mass closure using loop polyglyconate (Maxon; Davis and Geck, Gosport, UK) and surgical clips for skin.

Incision dimensions were measured with a flexible sterile ruler. The length of the skin incision at the end of the procedure was recorded as the wound length. The depth of the wound was assessed by measuring the total thickness of the abdominal wall. Wound blood loss during surgery was calculated by weighing swabs used exclusively while making the incision and closing the wound in all cases. No suction was used while making or closing the abdominal wound. Time taken to complete the laparotomy incision, from initial skin incision to complete opening of the peritoneum with total haemostasis, was recorded by an independent observer in the operating theatre. Other parameters, including total operative blood loss and total operating time, were recorded on a standardized form.

After operation all patients received morphine 1 mg/ml via patient-controlled analgesia (PCA) on the ward for up to 5 days, as required. No rectal or oral analgesia was given while PCA was in use. Wound-related pain scores (visual analogue scale)[6] and the number of days for which PCA was required for wound-related pain were recorded by blinded observers on the ward. These observers were members of the surgical team not involved with the procedure. Pain scores for the previous 24 h were recorded each morning after surgical rounds. Wound complications occurring at any stage after operation and at 1-month follow-up were recorded for all patients by independent observers blinded to the method of incision used. Wound infection was defined as the discharge of pus or fluid containing pathogenic organisms[7].

Table 1 Patient demographics

	Scalpel	Diathermy
No. of patients	50	50
Mean (range) age (years)	61 (15–84)	60 (32–85)
Sex ratio (M : F)	27 : 23	27 : 23
Malignant : benign pathology	33 : 17	32 : 18

Student's un-paired *t* test showed no significant differences between the groups

Statistical analysis

Results are presented as mean(s.e.m.). The unpaired Student's *t* test was used to assess patient parameters and measured operative parameters. The Mann–Whitney *U* test was used to assess postoperative pain scores. The level of statistical significance between groups was set at 5 per cent.

Results

Patient demographics

One hundred patients undergoing elective laparotomy were randomized prospectively to either scalpel or diathermy incision groups. There were no significant demographic differences between groups (*Table 1*). The mean(s.e.m.) age in the diathermy group was 61·0(2·0) years, compared with 60·1(2·2) years in the scalpel group. The indications for surgery in terms of underlying diagnosis did not differ significantly between groups, and a diagnosis of malignancy was present in the majority of patients in each group: diathermy 32 (64 per cent) of 50; scalpel 33 (66 per cent) of 50.

Operative parameters

The two groups did not differ significantly in terms of wound area, operating time or total operative blood loss (*Table 2*). Diathermy incisions caused significantly less blood loss than scalpel incisions (0·8(0·1) *versus* 1·7(0·3) ml/cm^2 respectively; $P = 0{\cdot}002$). Time taken for wound opening was significantly faster with diathermy than with the scalpel (6·1(0·4) *versus* 7·5(0·5) s/cm^2 respectively; $P = 0{\cdot}04$).

Postoperative pain

Postoperative pain, as assessed by visual analogue scale, was significantly reduced on the first ($P = 0{\cdot}04$) and second

($P = 0{\cdot}02$) postoperative days in the diathermy group compared with the scalpel group (*Table 3*). There was no significant difference in pain scores between the two groups on the third and subsequent postoperative days. Total postoperative PCA morphine requirement was significantly lower in the diathermy group than in the scalpel group (66(7·6) *versus* 92(9·1) ml respectively; $P < 0{\cdot}04$). The number of days for which PCA was required for wound-related postoperative pain was also significantly lower in the diathermy group than in the scalpel group (2·2(0·1) *versus* 2·6(0·1) days respectively; $P = 0{\cdot}01$).

Wound complications

The overall incidence of wound-related complications was low, and there was no significant difference in wound complication rate between the two groups before discharge or at 1-month follow-up. There were five postoperative wound infections: two in patients following diathermy incision and three after scalpel incision. All infected wounds responded to conservative treatment with intravenous antibiotics. There was one wound dehiscence in the diathermy group, in a patient receiving steroids before operation. This wound was subsequently re-explored and sutured in theatre, with no further wound-related complications. To date, one incisional hernia has been detected on follow-up, in a patient who underwent diathermy incision.

Table 2 Peroperative parameters measured

	Scalpel	Diathermy	P*
Incision time (s)	509(25)	469(21)	0·36
Wound area (cm^2)	78·8(5·4)	86·4(4·8)	0·30
Wound-related blood loss (ml)	105·5(8·7)	64·4(6·7)	0·0003
Incision time (s/cm^2)	7·5(0·5)	6·1(0·4)	0·04
Blood loss (ml/cm^2)	1·7(0·3)	0·8(0·1)	0·002
Total blood loss (ml)	915(88)	927(110)	0·93

Values are mean(s.e.m.).*Student's un-paired t test

Table 3 Postoperative pain scores and patient-controlled analgesia parameters

	Scalpel		Diathermy		
	Mean(s.e.m.)	Median (range)	Mean	Median (range)	P
Pain score					
Day 1	2·7(0·1)	3 (1–4)	2·3	2 (1–4)	0·04*
Day 2	2·4(0·1)	2·4 (1–4)	2·0(0·1)	2 (1–4)	0·02*
Day 3	1·9(0·1)	2 (1–3)	1·6(0·1)	2 (0–3)	0·15*
Day 4	1·4(0·1)	1 (0–3)	1·3(0·1)	1 (0–3)	0·34*
Day 5	1·2(0·1)	1 (0–2)	1·1(0·1)	1 (0–3)	0·43*
PCA					
Volume (ml)	92(9·1)		66(7·6)		0·036†
Days required	2·6(0·1)	3 (2–4)	2·2(0·1)	2 (1–4)	0·011†

PCA, patient-controlled analgesia. *Mann–Whitney U test, †Student's un-paired t test

Discussion

Before the introduction of non-explosive anaesthetic agents, electrosurgical instruments had only limited usage. Since the advent of halothane, diathermy has been used increasingly for haemostasis, and to a lesser extent for tissue dissection. Despite this, few surgeons use diathermy to incise skin. This reluctance to incise skin with diathermy is partly attributable to the belief that electrosurgical instruments increase devitalized tissue within the wound, which consequently leads to increased wound infection, increased scar formation and delayed wound healing. Early studies with primitive diathermy machines suggested that electrosurgical incisions were associated with just such charring and poor wound healing[8]. However, the development of oscillator units capable of delivering pure sinusoidal current has generated renewed interest in electrosurgery.

Subsequent animal studies suggested increased wound infection rates but no difference in wound bursting strengths[9,10]. Johnson and Serpell[2] demonstrated no difference between diathermy and scalpel in either wound inflammation or infection rate; however, they did not use diathermy for every layer of the incision. Other studies have demonstrated significant advantages to the use of electrosurgical incision, including shorter operating time, reduced postoperative pain and sealing of lymphatics during excision of malignant tumours[8]. Previous work by Dixon *et al.*[1] has shown that diathermy incision is more rapid than scalpel incision, although these authors failed to demonstrate a difference in postoperative pain scores between groups. In their study, however, pain was assessed only in the herniorrhaphy group and not in patients having the relatively more painful cholecystectomy incision. This latter group was subsequently shown by Hussain and Hussain[3] to have less postoperative pain following diathermy incision.

This study demonstrates that diathermy incision of all layers of a midline laparotomy wound was faster and associated with less blood loss than the equivalent scalpel incision. Diathermy incisions were associated with significantly lower postoperative pain scores in the first 48 h and with an overall decrease in PCA morphine volume requirements and duration of treatment. There was no significant difference in wound or peroperative complication rates between the two groups. The wound infection rate in both study groups was in line with rates previously reported from this department, and was attributed to the use of prophylactic antibiotics and the impervious wound-edge protector[5]. One patient required surgical wound revision because of dehiscence, although tissue-healing properties may have been inhibited by the preoperative use of corticosteroids in this patient.

These data demonstrate a significant advantage for the exclusive use of electrocautery in creating a midline abdominal incision in surgical patients. The traditional fears of excessive tissue devitalization and poor wound healing were not reflected in this study. Furthermore, the recent increase in bloodborne diseases such as hepatitis C and human immunodeficiency virus infection makes exclusion of the scalpel from the operative field an attractive option. It is proposed that electrosurgical incision of all abdominal layers is a safe and expedient technique that may be particularly useful in patients requiring a rapid incision or those with a bleeding diathesis.

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Randomized clinical trial

Randomized trial of needlescopic *versus* laparoscopic cholecystectomy

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Background: Several studies have reported the feasibility of using 'needlescopic' instruments with a diameter less than 3 mm in minimally invasive surgery. This study reports a comparison of needlescopic cholecystectomy and laparoscopic cholecystectomy.

Methods: Seventy-five patients with symptomatic chronic cholelithiasis were randomized to needlescopic ($n = 37$) or laparoscopic ($n = 38$) cholecystectomy.

Results: The duration of surgery in the two groups was similar. Patients in the needlescopic group had less pain (mean visual analogue score 2·2 *versus* 3·6; $P < 0{\cdot}003$) and had smaller scars (median length 17·0 *versus* 25·0 mm; $P < 0{\cdot}001$). In addition, patients in the needlescopic group tended to require fewer intramuscular pethidine injections ($P = 0{\cdot}05$). However, oral analgesic requirements in the two groups were similar. There were no complications in either group.

Conclusion: Needlescopic cholecystectomy resulted in less postoperative pain and a smaller surgical scar than laparoscopic cholecystectomy in patients with chronic cholecystitis.

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Introduction

Since the early 1990s, laparoscopic cholecystectomy has overtaken open cholecystectomy as the procedure of choice for chronic cholecystitis, offering patients a shorter hospital stay, less postoperative pain and an early recovery[1].

Needlescopic surgery, using instruments with a diameter less than 3 mm, has been adapted to perform laparoscopic cholecystectomy[2]. The small-calibre instruments result in smaller scars. This paper reports a randomized trial of needlescopic *versus* laparoscopic cholecystectomy in which duration of operation, postoperative pain and complications were compared in patients with symptomatic cholelithiasis.

Patients and methods

Consecutive patients with symptomatic cholelithiasis were included in the study. Patients with acute cholecystitis, as determined from clinical findings of acute peritonitis and ultrasonographic features of a thickened gallbladder wall with pericholecystic fluid collection, and patients with previous upper abdominal surgery were excluded from the trial. Four surgeons with individual experiences of more than ten needlescopic cholecystectomies participated in the trial.

Patients were randomized to receive needlescopic or laparoscopic cholecystectomy using a blind envelope system. Informed consent was obtained from every patient. The patients were informed of the type of procedure only after postoperative pain scores had been obtained.

The laparoscopic technique has been described elsewhere[3], and included a 10-mm umbilical port and three 5-mm working ports. For the needlescopic procedure, the ports were 10 mm, 2 mm, 3 mm and 2 mm in the umbilicus, right lateral abdomen, right hypochondrium and epigastrium respectively. The gallbladder fundus was held with a 2-mm grasper (USSC, Norwalk, Connecticut, USA), Hartmann's pouch was held with a 3-mm grasper (Storz, Tuttlingen, Germany) and dissection was done using a 1·7-mm ball-tipped coagulator (MIST, Smithfield, North Carolina, USA). The camera was changed to a 3-mm needlescope (Storz) for clipping of the cystic artery and cystic duct, and subsequently changed back to the 10-mm instrument for division of the cystic structures using a pair of 2-mm scissors (USSC). All wounds were infiltrated with 0·5 per cent bupivacaine in both groups. The combined lengths of the incisions were measured and dressings were the same in all patients.

All patients received a standard analgesic protocol with oral naproxen 375 mg twice daily and intramuscular

Table 1 Clinical details

	Needlescopic cholecystectomy (*n*=37)	Laparoscopic cholecystectomy (*n*=38)	*P*
Age (years)*	49	52	
Sex ratio (M : F)	14 : 23	17 : 21	
Duration of operation (min)*	50·0 (5·4)	45·0 (4·3)	0·29
Pain score†‡	2·2(1·5)	3·6(1·9)	< 0·003
Doses of naproxen*	2·0 (1·4)	2·0 (1·4)	0·45
Scar size (mm)*	17·0 (1·5)	25·0 (0·8)	< 0·001
Hospital stay (days)*	1 (1)	1·5 (1)	0·14

*Values are median (interquartile range); †values are mean(s.d.). ‡Visual analogue scale

pethidine 1 mg/kg every 6 h according to individual need. An independent observer, who was blinded to the type of operation, obtained a visual analogue score (range from 1 (minimal pain) to 10 (maximum pain)) the morning after surgery. All patients were reviewed after 2 weeks.

A mixed effects linear model was used to compare the two groups. Data were analysed using the SPSS 9.0 statistical software (SPSS, Chicago, Illinois, USA). The observed differences were assumed to be statistically significant if the probability of chance occurrence was $P < 0{\cdot}05$.

Results

A total of 75 patients entered the trial, 37 patients in the needlescopic group and 38 in the laparoscopic group. The results are summarized in *Table 1*. All patients were under the care of one of the four surgeons. During the study interval, 12 patients declined to be involved and five patients who had acute cholecystitis were excluded.

Four patients in the needlescopic group required conversion of the procedure, three to laparoscopic cholecystectomy and one to an open operation, because of adhesions or a thick-walled gallbladder. The conversion rate was 11 per cent. All four patients were included in the needlescopic group. There was one conversion to open surgery in the laparoscopic group. There was no difference in the median duration of surgery between the two groups (50 *versus* 45 min). There was also no difference in duration between the surgeons.

Patients who had needlescopic cholecystectomy had less postoperative pain according to mean visual analogue scores (2·2 *versus* 3·6; $P < 0{\cdot}003$). Although intramuscular pethidine requirement only reached borderline significance, the needlescopic group tended to require fewer injections than the laparoscopic group (total seven *versus* 12 injections; $P = 0{\cdot}05$).

The scars after needlescopic surgery were 32 per cent smaller than those after laparoscopy (median 17 *versus* 25 mm; $P < 0{\cdot}001$). There was no difference in duration of hospital stay and most patients were discharged within 2 days of surgery.

Clinical follow-up in outpatients was achieved in 96 per cent of patients and averaged 6 months (mean). There were no complications in either group.

Discussion

This randomized trial compared needlescopic with laparoscopic cholecystectomy. Two other retrospective studies have compared the two procedures[4,5]. One study showed a reduction in scar size for the needlescopic group while the other showed a decrease in the need for postoperative analgesia despite a longer operating time. Needlescopic instruments have been used to perform other operations such as appendicectomy, splenectomy, fundoplication, adrenalectomy, inguinal herniorrhaphy and thoracic sympathectomy[5–8].

A randomized trial comparing the use of 5- and 10-mm epigastric ports for laparoscopic cholecystectomy showed no reduction in pain scores and postoperative analgesic use[9]. The present study showed a lower pain score even with a reduction from 5-mm to 2- and 3-mm ports. Comparing 10-mm ports and 'needleports' could make a bigger difference to pain scores.

The reduction in postoperative pain in the needlescopic group may have resulted from the smaller wounds. The median scar length was used because a mean value would have skewed the results because of the inclusion of single conversions to open surgery in both groups.

The benefits to the patient have to be weighed against the additional expense involved in the use of needlescopic instruments. The needlescopic equipment, although purchased at additional cost, was reused at subsequent surgery. The thinner 2-mm graspers are more flexible than the 3-mm instruments and are liable to bend with rough handling, so retraction and dissection of the gallbladder was performed with the 3-mm grasper. In addition, non-thickened or non-inflammed gallbladders were selected for the study because needlescopic instruments are not as effective as larger instruments in handling a thickened gallbladder. The four patients in the needlescopic group whose operation was converted to laparoscopic cholecystectomy or open surgery remained in the original group for the purpose of analysis by intention to treat. The longer operating times in these patients did not affect the results of the needlescopic group.

Similar to a previous report[5], this study showed that the needlescopic procedure was no more difficult than the standard laparoscopic cholecystectomy. There was no difference in the duration of surgery and complications between the two groups. The dissection to identify the cystic structures was done safely using a 10-mm laparoscope. The view was changed to the 3-mm needlescope only for clipping, and not division, of the cystic artery and duct. Alternatively, the structures could be ligated with intracorporeal suturing without the need to change the laparoscope.

Needlescopic cholecystectomy resulted in less postoperative pain and a smaller scar than laparoscopic cholecystectomy. Needlescopic cholecystectomy appears to be as safe as laparoscopic cholecystectomy in patients with chronic cholecystitis.

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Original article

Incidence of benign lesions in patients resected for suspicious hilar obstruction

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Background: The differentiation between benign and malignant strictures at the hepatic hilum is difficult. The aim of this study was to assess the clinical and radiographical features of hilar lesions in a series of patients resected for a presumed cholangiocarcinoma.
Methods: Of 132 consecutive patients undergoing resection for a suspicious lesion at the hepatic hilum, 20 (15 per cent) had a histopathologically proven benign tumour, diagnosed as chronic fibrosing or erosive inflammation, sclerosing cholangitis, or a granular cell tumour. All medical files and radiological examinations of the patients were reassessed.
Results: Clinical symptoms were considered suspicious in 16 of 19 patients. At ultrasonography only one of 16 patients had an unsuspicious examination. In 14 of 18 patients cholangiographic images were considered suspicious. Overall reassessment confirmed a suspicious lesion in all 20 patients.
Conclusion: Because of the limitations of current diagnostic tools, a false-positive preoperative diagnosis of malignancy resulted in a 15 per cent resection rate of benign lesions in this series of suspicious hilar strictures.

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Introduction

Because of the incidence of benign lesions at the hepatic hilum, previous reports have cautioned against inappropriate surgical treatment of suspicious lesions in this area, without histological diagnosis[1–3]. An earlier study of the period from 1984 to 1990 found a 13 per cent false-positive rate for the preoperative diagnosis of malignancy in patients resected for a suspicious hilar obstruction.

The aim of this study was, first, to determine whether preoperative diagnosis and staging of hilar bile duct tumours after 1990 resulted in a reduction in the rate of false-positive diagnosis of malignancy and, second, to assess suspicious features of a hilar stricture during re-evaluation of all clinical and radiographical characteristics in these patients[4].

Patients and methods

Between 1983 and 1997, 132 consecutive patients underwent resection of a hilar stricture that was suspicious of a proximal bile duct carcinoma. In 20 patients (15 per cent), final histopathological assessment of the specimen showed a benign lesion. These patients (seven women and 13 men) had a median age of 50 (range 24–72) years. The clinical history and radiological findings in these 20 patients were reassessed.

The following combination of signs and symptoms was considered suspicious at presentation: jaundice, pruritus and/or discoloration of stools with symptoms of weight loss and abdominal pain. The combination of painless jaundice and weight loss was thought to be highly suspicious of malignancy.

When ultrasonography and/or computed tomography (CT) showed a tumour mass with extraductal extension at the hepatic confluence, the lesion was considered highly suspicious of malignancy. Stenosis and/or proximal (intrahepatic) bile duct dilatation without a tumour mass was also considered suspicious on ultrasonography and/or CT.

At cholangiography an irregular and eccentric stenosis and/or blunt ending was considered to be typical of a malignant lesion. In contrast, a smooth and concentric pattern and/or tapering of the duct was thought to be a feature of a benign lesion. With a combination of malignant and benign features, the lesion was considered suspicious.

Table 1 Preoperative findings in 20 patients with a benign hilar bile duct lesion who had resection for a presumed cholangiocarcinoma

	Suspicious	Not suspicious	Not available or not done
Clinical presentation	16	3	1
Ultrasonography	15	1	4
Computed tomography	4	5	11
ERCP or PTC	14	4	2
Doppler ultrasonography	6	10	4
Brush cytology	5	9	6
Laparoscopy	5	1	14

ERCP, endoscopic retrograde cholangiopancreatography; PTC, percutaneous transluminal cholangiography

At colour Doppler ultrasonography, increase of flow compatible with stenosis or absence of flow compatible with occlusion, as well as vascular stenosis or occlusion during visceral angiography, was considered suspicious of malignancy.

Results

Clinical presentation

Jaundice was present in 16 patients. Eleven patients suffered from upper abdominal pain. The pain, usually prominent in the right upper abdomen or epigastric region, was mostly vague and not persistent. Of 13 patients with retrievable weight recordings, 12 had weight loss of 3–15 (mean 7) kg.

Except for one patient who had a previous history of alcohol abuse, and who had also had hepatitis A 21 years earlier, no other relevant history was recorded. No patient had inflammatory bowel disease.

Overall, except for three patients who did not have jaundice as the first symptom, the combination of symptoms was considered to be suspicious of malignant disease in all other patients (suspicious in ten and highly suspicious in six) (*Table 1*).

Ultrasonography, computed tomography and Doppler flow assessment

In ten patients a tumour mass with extraductal extension at the hepatic hilum was shown on ultrasonography. In two patients CT showed a tumour mass. In only one patient was this mass also seen on ultrasonography. In contrast, a tumour mass was demonstrated on ultrasonography in three further patients, while on CT this could not be confirmed. In five patients a stenosis and/or proximal (intrahepatic) bile duct dilatation was seen during ultrasonography (and on CT in two patients). Mean tumour diameter at ultrasonography was 26 (range 15–40) mm. During colour Doppler assessment of the portal venous system and hepatic arterial system, increased flow was found in three patients and absence of flow in three, which was confirmed by angiography in one patient.

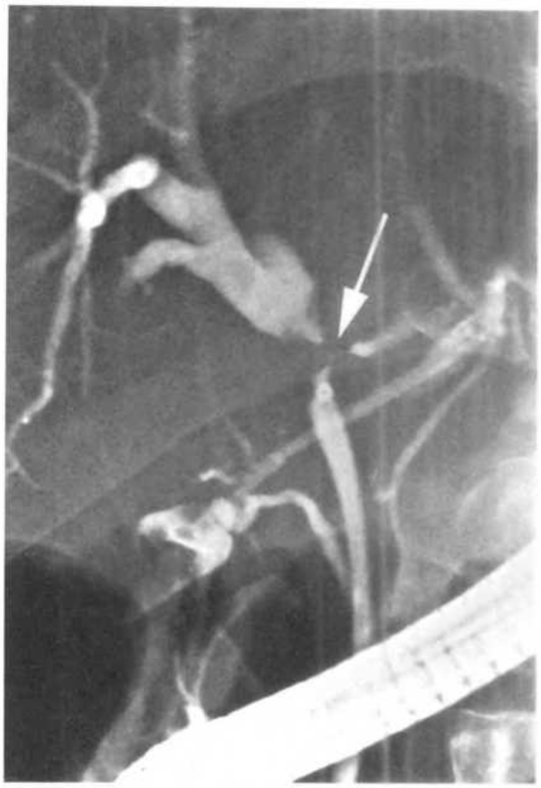

Fig. 1 Endoscopic retrograde cholangiopancreatography showing irregular stenosis with blunt ending at the site of the bifurcation with proximal dilatation, suggestive of a malignant lesion (arrow). Histopathological examination after resection showed a benign lesion (granular cell tumour)

Endoscopic retrograde cholangiopancreatography and percutaneous transluminal cholangiography

Of 16 endoscopic retrograde cholangiopancreatography (ERCP) procedures, three were considered as not suspicious and 13 as suspicious (*Fig. 1*). One of the patients who had undergone percutaneous transluminal cholangiography (PTC) had suspicious images. In total, 14 of all cholangiographs were considered as suspicious (*Table 1*).

Brush cytological examination revealed no malignant cells in nine patients. In four patients atypical cells were found, and in one patient brush cytology revealed cells highly suspicious for cholangiocarcinoma.

Diagnostic laparoscopy with intra-abdominal ultrasonography

Diagnostic laparoscopy with intra-abdominal ultrasonography was used in the last six patients, in four of whom a tumour mass was found at the hepatic hilum. In one patient, during intra-abdominal ultrasonography, there was suspicion of a gallbladder carcinoma with extension into the cystic duct. No diagnostic laparoscopic procedure revealed vascular involvement of the tumour.

Overall result of reassessments

Taking together the reassessments of clinical presentation, ERCP and PTC, ultrasonography (with Doppler flow assessment) and CT, angiography, diagnostic laparoscopy with intra-abdominal ultrasonography, and brush cytology, all 20 patients were classified as having suspicious lesions.

Intraoperative findings and postoperative histological examination

At laparotomy, an obviously suspicious tumour was found in 11 patients. In all other patients there was doubt as to whether the fibrosing lesion was malignant or benign. In two patients bile duct stones were found and in one patient Mirizzi syndrome was diagnosed at laparotomy[2].

All patients underwent local resection of the hepatic duct confluence to establish at least adequate biliary drainage, even when a lesion was thought to be benign during laparotomy. In three patients with a suspicious tumour, local resection was combined with an extended hemihepatectomy.

No biopsy specimens for frozen-section examination were taken before resection had been accomplished to avoid spilling tumour cells in the operative field. During resection, frozen-section examination of the surgical margins was performed to ensure a radical resection. In only one patient did examination of the proximal surgical margin reveal adenocarcinoma and, because it was impossible to resect more proximally into the liver, a presumed palliative local resection was performed. However, definitive pathological examination revealed only fibrosis.

In all three patients who had undergone liver resection, definitive histopathological examination of the liver specimen showed sclerosing cholangitis with concentric periductal fibrosis and focal formation of granulomatous tissue. In two patients histopathological findings were compatible with a granular cell tumour, with cells displaying granular cytoplasm[5]. In all patients the specimen showed fibrosis with non-specific, mild to moderate chronic inflammation, consisting of mixed cells (lymphocytes, granulocytes and plasma cells), sometimes with signs of erosion.

Discussion

Because of relative inexperience with benign lesions at the hepatic hilum and the inability to confirm these lesions with currently available preoperative diagnostic tools, the likelihood of undertreating or overtreating these patients is considerable. In this study the proportion of patients with a false-positive preoperative diagnosis of malignancy was comparable with previous results[1]. When focusing only on the period of the 1991–1997 study in which intra-abdominal ultrasonography was used routinely, nine patients with a benign lesion were found among 50 patients, showing that the incidence of diagnostic errors did not decrease. Consequently, despite the fact that two patients had a granular cell tumour requiring resection, seven patients underwent resection that was unnecessary, other than to establish at least adequate biliary drainage.

The issue is that, in the absence of previous biliary surgery or stones, any stenosis or tumour mass at the hepatic confluence should be considered malignant[1,2]. Application of improved diagnostic methods, such as intra-abdominal ultrasonography and thin-section multiphasic spiral CT and magnetic resonance cholangiopancreatography, potentially increases diagnostic accuracy, but whether these tools will improve the differentiation of benign and malignant lesions awaits further clinical assessment[6,7]. Preoperative histological or cytological examination by means of biopsy or brush cytology is often difficult, is liable to false-negative results and carries a potential risk of implantation metastases[2,4,8].

Careful review of all preoperative information and radiographic images confirmed the initial diagnosis. Therefore, the decision to undertake resection was not an error of judgement, but reflects a consistent policy in the management of proximal bile duct lesions. Even during laparotomy, in the majority of cases (11 of 20) the tumour was considered to be malignant. Obviously, resection of the lesion is still the most reliable way to rule out malignancy[8].

In conclusion, resection of a benign lesion mimicking a malignant stricture at the proximal bile duct cannot be avoided.

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Original article

The National Liver Transplantation Audit: an overview of patients presenting for liver transplantation from 1994 to 1998

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Background: The aim of this study was to describe current clinical practice in liver transplantation in the UK and Ireland, to provide overall 1-year graft and patient survival rates, and to study some preoperative risk factors.
Methods: All patients receiving a liver transplant in the UK or Ireland between 1 March 1994 and 30 September 1998 were included. Data were collected on patients at the time of transplantation, 3 months after grafting and annually thereafter until the patient's death. The main outcome measures were graft and patient survival at 1 year.
Results: A total of 3102 liver transplants were carried out, of which 87 per cent were first transplants. The mean age at first transplantation was 42 (range 0–76) years. The most common indications for transplantation were primary biliary cirrhosis, alcoholic cirrhosis and posthepatitis C cirrhosis, but variations existed between sexes and centres. Risk factors associated with lower graft and patient survival were the presence of acute disease, being transplanted from hospital, and the need for renal and/or ventilatory support before operation.
Conclusion: Donor and recipient demographics are consistent with data held by the European Liver Transplant Registry, as are 1-year graft and patient survival rates. Variation across centres in factors such as the primary indication for liver transplantation, population demographics, the clinical status of each patient, incidence of retransplantation and other risk factors contributes to the problem of adjusting for case mix.

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Introduction

Improvements in surgical technique, anaesthetic management, intensive care and immunosuppressive regimens have resulted in a significant increase in survival rates from liver transplantation. In 1980, the 1-year survival rate was approximately 30 per cent[1], whereas 10 years later the rate was more than 80 per cent[2,3] (international values). Transplantation can give patients with an extremely poor prognosis the opportunity of long-term survival and good quality of life[4].

The high financial costs associated with liver transplantation[5] and an increasing shortage of valuable donor organs demand that careful assessment of the results of operation is necessary to ensure optimal outcome[6]. The UK National Liver Transplantation Audit commenced in March 1994. All patients receiving a liver transplant in the UK or Ireland after this date were entered into a national database, and their outcome was monitored.

The audit was established to describe current clinical practice in liver transplantation for the eight liver transplant centres in the UK and Ireland, to compare outcome across centres and patient groups, and to develop statistical models to stratify patients according to risk of an adverse outcome. This paper describes the recipient and donor population of those undergoing liver transplantation since 1994, and describes 1-year graft and patient survival rates.

Patients and methods

Once a patient within the UK or Ireland is considered eligible for liver transplantation, he or she is registered with the UK Transplant Support Service Authority (UKTSSA). Data are collected by centres on all transplanted patients at the time of transplantation, 3 months postgrafting, and annually thereafter until the patient's death by means of detailed questionnaires. These data are maintained by the UKTSSA within a large national transplant database.

This paper reviews 1-year graft and patient survival for all patients who had transplantation between 1 March 1994 and 30 September 1998. Overall, 98 (range across centres 97–100) per cent of transplant-related forms and 96 (range 89–100) per cent of 1-year related forms were returned. The majority of 1-year forms not returned related to overseas patients, who are difficult to follow up.

Children were defined as patients aged 16 years or less at the time of transplantation. The main paediatric transplant centres are Birmingham Children's Hospital, King's College Hospital, London, and Addenbrooke's Hospital, Cambridge, accounting for 96 per cent of all paediatric cases. Graft failure was defined as retransplantation or patient death.

For the purpose of this analysis, the most recent data set was used, which was validated rigorously by the audit team. All data were subjected to computer-based validation, which highlighted any inconsistencies as well as missing or invalid data. An independent validation of 2 per cent (between ten and 18 patients at each centre) of patient notes was undertaken. For each variable, the overall percentage of missing data was: 0 per cent for date of birth, date of transplant, sex and graft number, 4 per cent for primary diagnosis, 7 per cent for ethnicity, 2 per cent for inpatient before operation, 4 per cent for preoperative renal support, 3 per cent for preoperative ventilatory support, 4 per cent for cause of failure, 0 per cent for date of failure and date of death, and 4 per cent for cause of death. The percentage of completed data validated as being correct, based on the 2 per cent sample, was: date of birth 100 per cent, date of transplant 100 per cent, sex 100 per cent, graft number 100 per cent, primary diagnosis 97 per cent, ethnicity 94 per cent, inpatient before operation 95 per cent, preoperative renal support 92 per cent, preoperative ventilatory support 97 per cent, cause of failure 93 per cent, date of failure 100 per cent, cause of death 100 per cent, date of death 100 per cent.

Statistical analysis

Analysis of the data set was performed with Statistical Analysis Software (SAS) (SAS Institute, Cary, North Carolina, USA). One-year graft and patient survival estimates and 95 per cent confidence intervals (c.i.) were calculated by means of the product limit (Kaplan–Meier) method.

Results

The number of patients who had a liver transplant during the study period totalled 3102, of which 2704 (87 per cent) were first transplants. The proportion undergoing retransplantation varied from 9 to 22 per cent between centres. The age at transplant and sex distribution of patients receiving their first liver transplant is given in *Fig. 1*.

The overall number of operations per year did not change during the study period, being just less than 600 per year in the UK and Ireland. Each centre carried out approximately the same number of transplants each year during the course of the study.

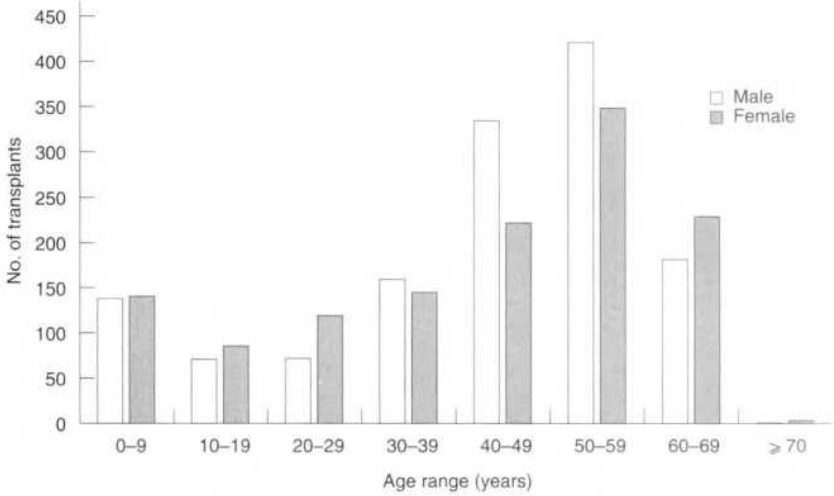

Fig. 1 Age and sex of first-transplant recipients (mean age 42 (range 0–76) years)

Most recipients were of blood group O and A (44 and 40 per cent respectively), with 11 per cent being of group B and 5 per cent of group AB. Eighty-nine per cent of all first transplants were blood group identical, 10 per cent were compatible and 1 per cent incompatible. The majority of incompatible transplants were of blood group A to a recipient of group O. *Table 1* shows the 1-year graft and patient survival estimates for each of the groups.

Table 1 One-year graft and patient survival rates following first transplant in various risk groups

Risk factor	Estimated 1-year graft survival (%)	Estimated 1-year patient survival (%)
Type of disease		
Chronic	77 (75–79)	83 (82–85)
Acute	65 (58–72)	70 (65–74)
Transplantation		
From home	79 (77–81)	85 (83–87)
From hospital	68 (64–71)	74 (70–77)
Ventilatory support		
No	77 (77–79)	83 (81–85)
Yes	62 (56–68)	68 (62–73)
Renal support		
No	77 (75–79)	83 (81–85)
Yes	65 (60–71)	70 (62–73)
Blood group		
Identical	76 (74–78)	82 (80–84)
Compatible	65 (59–72)	70 (63–76)
Incompatible	57 (35–78)	70 (50–90)
Not ventilated, no renal support	78 (75–80)	83 (82–85)
Ventilated, no renal support	70 (62–78)	75 (68–83)
Not ventilated, renal support	73 (66–80)	78 (71–84)
Ventilated, renal support	55 (47–64)	61 (53–70)

Values in parentheses are 95 per cent confidence intervals

Donor age and sex distribution of all transplant donors is illustrated in *Fig. 2*. The main causes of death amongst donors were: spontaneous intracranial haemorrhage (50 and 69 per cent of male and female deaths respectively), road traffic accidents (27 and 11 per cent) and other accidents (14 and 7 per cent). Of a total 603 road traffic accidents, 414 were in men aged less than 40 years. Fifty-two per cent of liver donors were blood group O; 38, 8 and 2 per cent were blood groups A, B and AB respectively.

Table 2 shows the distribution of primary liver diagnoses. There was a significant amount of variation between centres, as shown in *Table 3*, which gives the number of transplants carried out for each primary liver diagnosis as a percentage of the total transplants undertaken at each hospital. The same liver diagnosis groupings have been used as in *Table 2*. No consistent trends were seen in the distribution of primary liver diagnosis over the period of the study. There was some variation in primary liver diagnosis among patients of different ethnic origin (*Table 4*).

The clinical status of the 2704 first-transplant recipients was as follows: 19 per cent of patients had acute disease (as defined in *Table 2*), 35 per cent were in hospital immediately before transplantation, 16 per cent were on renal support (10 per cent haemodialysis and 6 per cent filtration) and 13 per cent were ventilated before operation. One-year graft and patient survival estimates in these risk groups are given in *Table 1*. There were no consistent trends in the proportion of patients with these risk factors over the study period.

The vast majority of transplants (88 per cent) were whole livers; this remained fairly constant over the course of the study. However, the use of split livers has increased slowly over the last five years, from 2 per cent of all transplants in

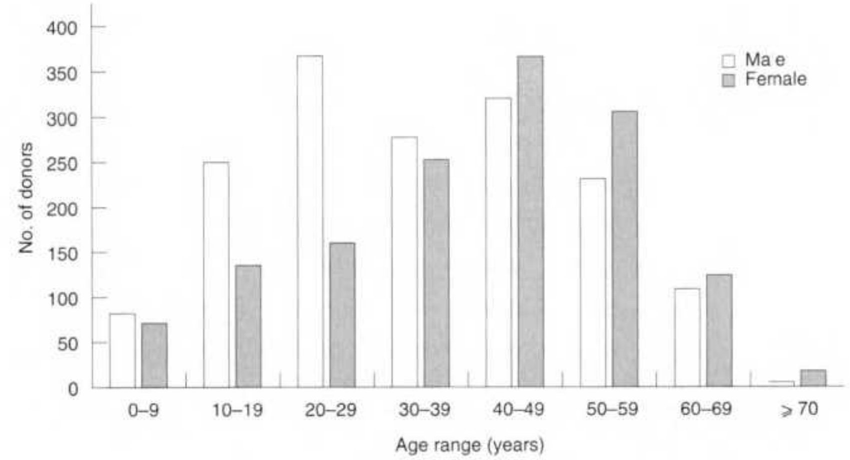

Fig. 2 Age and sex of all liver donors (mean age 37 (range 0–78) years)

Table 2 Primary indication for liver transplantation in 2692 patients (12 patients missing)

	Men (> 16 years)	Women (> 16 years)	Children (≤ 16 years)
Primary biliary cirrhosis	39	393	1
Autoimmune cirrhosis	34	71	7
Postviral cirrhosis			
Posthepatitis B cirrhosis	125	15	2
Posthepatitis C cirrhosis	196	87	4
Alcoholic cirrhosis	316	71	0
Acute disease			
Acute hepatitis: cause unknown	46	111	45
Acute hepatitis A	3	2	0
Acute hepatitis B	12	9	3
Acute Wilson's disease	5	7	8
Drug induced: paracetamol	31	90	8
Drug or toxin induced (not paracetamol)	10	20	8
Cancer			
Hepatocellular carcinoma			
Non-cirrhotic	7	4	1
Cirrhotic	23	3	1
Cholangiocarcinoma	4	6	0
Secondary liver tumour	7	8	0
Other hepatic malignancy	6	5	12
Biliary atresia and variants			
Biliary atresia	0	1	120
Congenital biliary disease	1	9	8
Cholestatic disease (paediatric)	0	0	21
Primary sclerosing cholangitis	143	54	8
Metabolic			
α_1-Antitrypsin deficiency	15	4	27
Chronic Wilson's disease	7	6	5
Metabolic disease	22	12	30
Haemochromatosis	12	2	0
Other			
Budd–Chiari syndrome	4	17	1
Secondary biliary cirrhosis	11	17	4
Cryptogenic cirrhosis	77	47	12
Benign liver tumour	1	1	3
Polycystic disease	1	12	1
Other, not already noted	34	23	42
Unknown	7	3	1
Total	1199	1110	383

Table 3 Primary indication for liver transplantation at each centre

	Centre							
	A	B	C	D	E	F	G	H
Primary biliary cirrhosis	9	34	12	15	21	21	20	13
Autoimmune cirrhosis	2	4	3	4	5	9	9	3
Viral cirrhosis	20	4	33	10	11	9	4	21
Alcoholic cirrhosis	10	17	22	15	11	26	15	16
Acute disease	18	19	9	21	16	13	17	9
Cancer	3	1	3	7	2	6	1	3
Biliary disease and variants	12	0	0	1	8	0	0	4
Primary sclerosing cholangitis	4	12	8	8	9	7	15	7
Metabolic	7	1	4	4	7	2	6	5
Other	14	9	6	15	9	8	14	20

Values are percentages

Table 4 Primary indication for liver transplantation by ethnicity in 2513 patients (191 missing)

	White (*n*=2216)	Asian (*n*=209)	Black (*n*=45)	Other (*n*=43)
Primary biliary cirrhosis	396 (18)	10 (5)	1 (2)	2 (5)
Autoimmune cirrhosis	100 (5)	4 (2)	1 (2)	1 (2)
Viral cirrhosis	289 (13)	79 (38)	11 (24)	21 (49)
Alcoholic cirrhosis	346 (16)	14 (7)	1 (2)	0 (0)
Acute disease	339 (15)	31 (15)	15 (33)	3 (7)
Cancer	70 (3)	5 (2)	1 (2)	2 (5)
Biliary atresia and variants	112 (5)	19 (9)	3 (7)	8 (19)
Primary sclerosing cholangitis	182 (8)	7 (3)	5 (11)	1 (2)
Metabolic	115 (5)	14 (7)	1 (2)	3 (7)
Other	267 (12)	26 (12)	6 (13)	2 (5)

Values in parentheses are percentages

Table 5 Cause of failure of first graft against time of failure post-transplant

	Time after transplant							
	1 week	2 weeks	1 month	3 months	1 year	> 1 year	Total	%
Acute rejection								
Retransplanted	1	5	0	0	0	0	6	4
Not retransplanted	0	7	1	0	0	1	9	
Chronic rejection								
Retransplanted	0	0	0	10	36	17	63	22
Not retransplanted	0	0	0	2	16	9	27	
Primary non-function								
Retransplanted	26	2	1	0	0	0	29	10
Not retransplanted	10	2	2	0	0	0	14	
Vascular occlusion								
Retransplanted	8	10	21	12	5	1	57	17
Not retransplanted	2	1	5	2	1	1	12	
Non-thrombotic infarction								
Retransplanted	1	6	1	3	1	0	12	6
Not retransplanted	2	6	1	2	0	0	11	
Recurrent disease								
Retransplanted	0	0	0	0	6	1	7	8
Not retransplanted	0	0	2	1	12	11	26	
Biliary complications								
Retransplanted	0	0	1	1	7	0	9	4
Not retransplanted	0	0	2	2	1	2	7	
Other								
Retransplanted	3	0	4	4	2	8	21	13
Not retransplanted	9	11	3	3	7	1	34	
Unknown								
Retransplanted	2	1	3	3	4	8	21	16
Not retransplanted	13	3	5	11	7	6	45	

1994 to 6 per cent in 1998. The use of split and reduced livers was concentrated in the centres that transplant the majority of children. Only 17 transplants of the cohort were heterotopic in nature.

Of the 2704 first transplants, 436 subsequently failed during the study period, resulting in either retransplantation or patient death. A further 213 patients died during the course of the study from causes unrelated to graft failure. The most common causes of graft failure were chronic rejection (22 per cent), vascular occlusion (17 per cent) and primary non-function (10 per cent). Some 245 of these 436 patients with graft failure were retransplanted within the study period. The causes of graft failure and the interval between transplantation and failure (for first grafts only) are given in *Table 5*.

For second transplants, 19 per cent were in patients who originally presented with acute disease, 68 per cent were in hospital before operation, 26 per cent were on renal support (13 per cent haemodialysis and 13 per cent haemofiltration) and 26 per cent were ventilated. Eighty-two per cent of second grafts were blood group identical, 16 per cent were compatible and 2 per cent incompatible. Again, the majority of incompatible transplants were of blood group A to a recipient of group O. Overall 1-year graft survival rates are 56 (95 per cent c.i. 50–62) per cent for second transplants, compared with 75 (73–77) per cent for first transplants. The corresponding patient survival rates are 65 (95 per cent c.i. 60–71) per cent compared with 81 (79–83) per cent.

The most common cause of death was septicaemia (20 per cent). Twenty-one patients (1 per cent) undergoing first transplantation died at operation. For second transplants (326 cases), five patients (2 per cent) died at operation. The main causes of death in these patients were cardiac or pulmonary complications. The 30-day in-hospital mortality rate among patients receiving their first transplant (patients who died within 30 days of transplant who had not left hospital and who had not been retransplanted) was 8 per cent (218 patients). The overall in-hospital mortality rate for first transplants (patients who died having been admitted for a first transplant and who never left hospital) was 13 per cent (341 patients).

Discussion

These data show that there is an equal mix of male and female liver transplant recipients, with slightly more males being transplanted in the age group of 30–60 years, but slightly more females in all other age groups. This is consistent with information from the European Liver Transplant Registry. The most obvious reason for this difference is that the liver diseases more common in men (alcoholic cirrhosis, posthepatitis C cirrhosis and postviral cirrhosis) are likely to cause liver failure in the younger 'middle ages'; diseases that are more common in women (posthepatitis B cirrhosis and acute hepatitis) tend to occur in young women (in the case of acute hepatitis) or, in the case of posthepatitis B cirrhosis, ultimately cause liver failure in older women after they have had the disease for 10–20 years.

Although the number of liver transplants performed in the UK increased steadily during the 1980s and early 1990s[7], the number of operations carried out per year during the course of the audit remained steady. A possible reason for this is a limited donor pool.

There are more male liver donors than female, primarily because of an excess number of male deaths in the younger age group, largely from road traffic accidents. In contrast, spontaneous intracranial haemorrhage is more common in the older age group. In this donor cohort, there were more female than male deaths as a result of intracranial haemorrhage, which accounts for the excess of female donor deaths in the upper age groups.

The variation between centres in primary indication for liver transplantation highlights the difficulty in making comparisons between centres. Some differences in the distribution of primary indication across centres can be accounted for by the variation in prevalence of disease in the catchment area, the population demographic profile, whether or not a centre specializes in paediatric liver transplantation, and referral patterns. Variation in these factors contributes to the generic problem of adjusting for case mix in order to compare performance across centres or to produce prognostic estimates for diagnostic subgroups. Accepting that details of case mix in different countries have not been taken into account, the UK liver transplant programme has had overall graft and patient survival rates entirely consistent with the experience of liver transplantation in the rest of Europe during this study period.

Table 5 illustrates that the prospect of retransplantation following graft failure is partly dependent on the cause, but not the timing, of graft failure. Nearly all patients whose graft failed as a result of vascular occlusion were retransplanted. Most patients who suffered from chronic rejection or primary non-function were retransplanted. In contrast, less than one-quarter of those who suffered recurrent disease had a second transplant.

Graft survival following retransplantation is well known to be lower than that associated with the first transplant[8], although outcome has improved in recent years[9], especially in the case of 'elective' retransplants[10]. These data show that patients undergoing retransplantation are more likely to have associated risk factors (renal support, ventilatory support, or to be in hospital before operation) than those undergoing primary grafting.

The procedure of reduced liver grafting[11] was introduced primarily in response to the shortage of donor organs of appropriate size, especially in paediatric liver transplantation. As can be seen from *Figs 1* and 2, there is a shortage of very young donors, but a slight surplus of donors in the age group 10–40 years. Because of the lack of size-matched grafts in the infant population, the pretransplant mortality rate has historically been reported as 25–50 per cent[12]. The obvious disadvantage of reduced grafting procedures is that they do not expand the total donor pool. Split grafting techniques[13] overcome this disadvantage, and may even render reduced liver grafting obsolete[14] over the coming years. These data show that, while the use of whole livers was constant over the cohort period, split grafting increased, primarily at the expense of transplanting reduced livers.

This paper has looked briefly at some recipient risk factors for liver transplantation; these are the presence of acute disease, inpatient before operation, ventilatory support, renal support and retransplantation. Other possible confounding factors were not considered. A further intention of this audit is to carry out a detailed risk analysis of donor, recipient and operative variables, to quantify the risk associated with each variable. It is recognized that there is a need for unified criteria for collecting data[15] in order to allow the comparison of results from different centres, each of which will have its own patient case mix and postoperative management procedures. The risk analysis will allow the development of a model to adjust substantially for differences in case mix, and therefore to make more valid comparisons between centres. Decisions about liver allocation clearly depend on many additional factors. The role of prognostic modelling in assisting such decisions requires further investigation.

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Original article

Experimental study of a type 3 phosphodiesterase inhibitor on liver graft function

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Background: The number of liver transplant recipients is increasing but donor organ shortages have become more severe. The effect of milrinone, a type 3 phosphodiesterase inhibitor (PDEI), on non-heart-beating donor grafts was evaluated using an orthotopic liver transplantation model in rats.

Methods: Type 3 PDEI or normal saline (control group) was given intravenously to the donor animals for 60 min continuously ($50\,\mu g\,kg^{-1}\,min^{-1}$) before 60 min of warm ischaemia followed by cold preservation and subsequent transplantation. Survival, serum chemistry, bile output, histopathological findings and tissue cyclic 3′,5′-adenosine monophosphate (cAMP) concentrations were then compared.

Results: Five of seven animals in the PDEI group were alive at 7 days, compared with only one of seven rats in the control group ($P < 0.01$). Serum levels of alanine aminotransferase 2 and 6 h after reperfusion, and hyaluronic acid levels 6 h after reperfusion, were significantly lower in the PDEI group than in the control group. Bile output from the transplanted graft was significantly greater in the PDEI group than in controls 2 h after reperfusion ($P < 0.01$). The mean necrotic area 6 h after reperfusion was also reduced in the PDEI-treated grafts ($P < 0.01$). cAMP levels in liver tissue at the end of both warm and cold ischaemia, and 2 and 6 h after reperfusion, were significantly higher in the PDEI group compared with those in the control group.

Conclusion: Type 3 PDEI attenuated the graft injury caused by warm and cold ischaemia and subsequent reperfusion injury via an increase in intracellular cAMP levels. This treatment may be a novel pharmacological intervention for safe and efficient usage of liver grafts from non-heart-beating donors.

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Introduction

Liver transplantation has become a widely accepted treatment for patients with terminal liver disease[1]. With the annual increase in the number of liver transplant recipients on waiting lists, donor organ shortages have become more severe[1,2]. One possible strategy for increasing the organ procurement rate and alleviating organ shortages is the use of liver grafts from non-heart-beating donors[1,3–5]. Nathan *et al.*[6] have reported that an increase of 20–25 per cent in organ donors could be achieved if non-heart-beating donors were used routinely. However, clinically successful transplants have occurred only in cases where the grafts were procured after short periods of warm ischaemia in a controlled operating room setting[3,4]. For safe and efficient usage of liver grafts from non-heart-beating donors, novel pharmacological interventions are still necessary.

Type 3 phosphodiesterase inhibitors (PDEIs) such as amrinone and milrinone have recently been used in the field of cardiology for their inotropic and vasodilating properties to treat heart failure[7–9]. Type 3 PDEI inhibits type 3 phosphodiesterase, which hydrolyses adenosine 3′,5′-cyclic monophosphate (cAMP) into adenosine 5′-monophosphate, and increases intracellular cAMP concentrations[7–9]. It has been reported that increased cellular cAMP content also exerts beneficial effects on hepatic ischaemia–reperfusion injury[10,11].

The aim of this study was to investigate whether milrinone, a type 3 PDEI, would improve the function of livers harvested from non-heart-beating donors in rats.

Materials and methods

Experimental design

Animals

Male Lewis rats weighing 190–210 g (Seac Yoshitomi, Fukuoka, Japan) were used. The rats were allowed free access to water and standard laboratory chow, and were kept under controlled environmental conditions. Before being operated on, the rats were fasted for 12 h and allowed free access only to water. The Kyushu University Institutional Animal Care and Use Committee approved all animal protocols according to published criteria[12].

Orthotopic liver transplantation

Orthotopic rat liver transplantation with arterial reconstruction was performed using the cuff technique described by Kamada and Calne[13], with minor modifications. Under ether anaesthesia, laparotomy was performed and the liver was skeletonized. For biliary reconstruction an internal stent tube (22-G Angiocath; Becton Dickinson, Sandy, Utah, USA) was used. As a model of liver transplantation from non-heart-beating donors, warm ischaemia for 60 min was induced by clamping the portal vein and the hepatic artery of donor rats. Immediately before the portal vein was clamped, the donor rat received 100 units heparin through the penile vein. After 60 min of warm ischaemia, the liver was flushed with chilled lactated Ringer's solution via the abdominal aorta, and then harvested and stored at 4°C in lactated Ringer's solution for 45 min. The suprahepatic vena cava was anastomosed with 7/0 continuous polypropylene suture (Prolene; Ethicon, Somerville, New Jersey, USA), and portal vein, infrahepatic vena cava and hepatic artery reconstruction was performed by means of the cuff technique (6- and 8-Fr intravenous cannulas; Portex, Hythe, UK). Arterial reconstruction was carried out under microscopy using a cuff (24-G Angiocath; Becton Dickinson) technique. Biliary reconstruction using an internal stent was also carried out under microscopy. The cold ischaemic time and anhepatic time were intentionally fixed at 45 and 15 min respectively.

Experimental groups

According to the donor treatment, the recipient animals were classified into two groups. In the PDEI group ($n = 17$), PDEI (milrinone; Yamanouchi, Tokyo, Japan) was administered continuously at a rate of $50\,\mu g\,kg^{-1}\,min^{-1}$. In the control group ($n = 17$), normal saline was administered at the same rate using a syringe pump (Model TE311; Terumo, Tokyo, Japan) via a catheter (2-Fr intravenous cannula; Portex) placed in the donor left jugular vein for 60 min before the induction of warm ischaemia. To determine the survival rate, seven rats in each group were monitored for 7 days after operation.

Sample collection

Five rats in each group were killed 2 and 6 h after reperfusion for the determination of serum chemistry and tissue cAMP levels. For bile collection, a micro-silicone rubber tube (inside diameter 0·3 mm, outside diameter 0·5 mm; Isolier und Messtechnik, Berlin, Germany), 25 cm in length, was placed in the common bile duct. The total bile output through the catheter at 1 and 2 h after reperfusion was measured, and values were expressed as bile output (µl) per 100 g body-weight per h. For the determination of hepatic cAMP levels, five rats were killed at the end of 60 min of warm ischaemia, and five rats were killed at the end of 45 min of cold ischaemia. Five normal rats were also killed for determining normal hepatic cAMP levels. Blood samples were drawn from the abdominal aorta, centrifuged immediately at 3000 r.p.m. at 4°C for 10 min, and then stored at −80°C. The liver tissue samples for the cAMP assay were immediately immersed in liquid nitrogen and stored at −80°C. Liver specimens for the histological studies were fixed in 10 per cent neutral buffered formalin.

Biochemical measurements

Alanine aminotransferase (ALAT) and hyaluronic acid were assayed using standard enzymatic methods.

Histopathological examinations

Formalin-fixed liver specimens were embedded in paraffin and 3-µm sections were stained with haematoxylin and eosin. The images of five separate stained slices of the liver 6 h after reperfusion were obtained as PICT files with a FUJIX digital camera HC 2500 3CCD and Photograb 2500 software (Fuji, Tokyo, Japan). The necrotic and total areas of each liver section were calculated using NIH image analysis software (Wayne Rasband, National Institutes of Health, Bethesda, Maryland, USA), and the fraction of necrotic area compared with the total area of the liver section was determined.

Hepatic cyclic 3′,5′-adenosine monophosphate assay

The liver samples for the cAMP assay were weighed and homogenized with 6 per cent trichloroacetic acid. After the removal of the trichloroacetic acid with water-saturated ether, tissue cAMP levels were determined from aliquots of the neutralized supernatant using an enzyme immunoassay kit (Biotrak cAMP system; Amersham Pharmacia Biotech, Little Chalfont, UK).

Statistical analysis

All values were expressed as mean(s.d.). The Mann–Whitney *U* test was applied for data analysis. The animal survival rate was determined with the Kaplan–Meier method, using the log rank test to determine significance. $P < 0.05$ was considered statistically significant.

Results

Survival rate

Fig. 1 shows the survival rate after liver transplantation with the grafts from non-heart-beating donors. Only one of seven animals with a saline-treated graft, compared with five of seven with PDEI-treated grafts, was alive at 7 days ($P < 0.01$).

Bile output

Table 1 gives the changes in bile output 1 and 2 h after reperfusion. Compared with the control group, animals in the PDEI group produced significantly larger amounts of bile 2 h after reperfusion ($P < 0.01$).

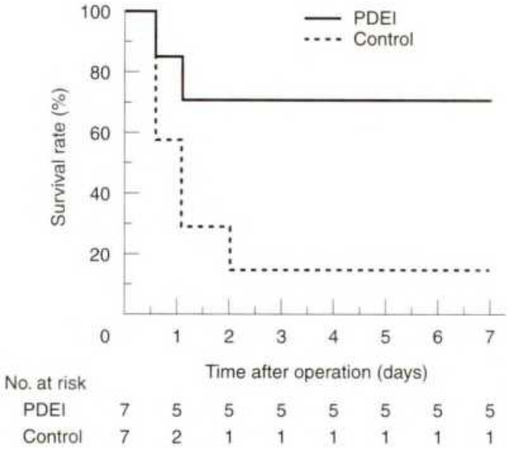


Fig. 1 Effects of donor treatment with type 3 phosphodiesterase inhibitor (PDEI) on the survival of recipient animals. $P < 0.05$ (log rank test)

Table 1 Bile production after reperfusion

	Time after operation	
	1 h	2 h
Bile output (µl per 100 g body-weight per h)		
Control	0·16(0·10)	0·33(0·25)
PDEI	0·48(0·34)	1·18(0·17)*

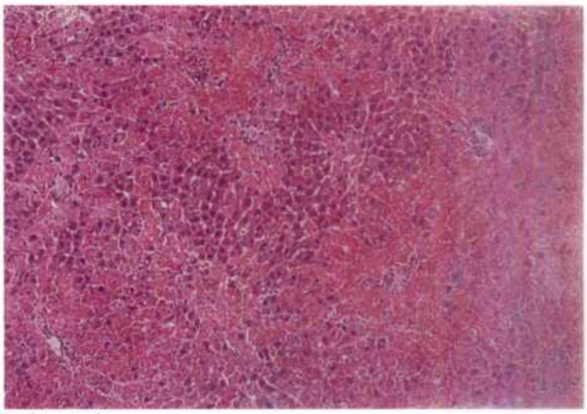
Values are mean(s.d.). PDEI, phosphodiesterase inhibitor. $^*P < 0.01$ *versus* control at 2 h (Mann–Whitney *U* test)

Serum chemistry data

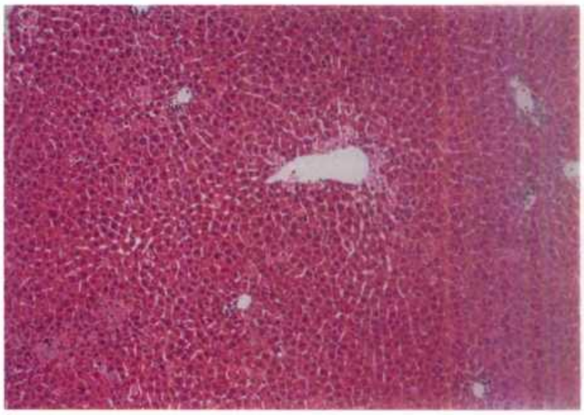
As shown in *Table 2*, serum ALAT levels 2 and 6 h after reperfusion, and the serum hyaluronic acid level 6 h after reperfusion, were significantly lower in the PDEI group than in the control group.

Histological assessment of hepatocellular necrosis

Fig. 2 shows the most representative haematoxylin and eosin-stained section of the liver graft 6 h after reperfusion in control and PDEI groups. Massive haemorrhagic necrosis was observed in the control group (*Fig. 2a*), but not in the PDEI group (*Fig. 2b*). The mean(s.d.) portion of necrotic area, which was calculated using an image analyser system, was 12·4(6·1) per cent in the PDEI group and 33·3(12·2) per cent in controls ($P < 0.01$).



a Control



b PDEI group

Fig. 2 Haematoxylin and eosin-stained section of a liver graft 6 h after reperfusion from an animal in **a** the control group and **b** the phosphodiesterase inhibitor (PDEI) group. Massive haemorrhagic necrosis is present in the control, but is less significant in the PDEI-treated graft

Table 2 Serum alanine aminotransferase and hyaluronic acid levels following reperfusion

	Time after operation	
	2 h	6 h
ALAT (units l^{-1})		
Control	9001(1670)	14 834(6936)
PDEI	5031(838)*	5 983(1408)†
Hyaluronic acid (ng ml^{-1})		
Control	77(36)	200(91)
PDEI	68(24)	74(25)†

Values are mean(s.d.). ALAT, alanine aminotransferase; PDEI, phosphodiesterase inhibitor. $^*P < 0.01$, $^\dagger P < 0.05$ *versus* control (Mann–Whitney *U* test)

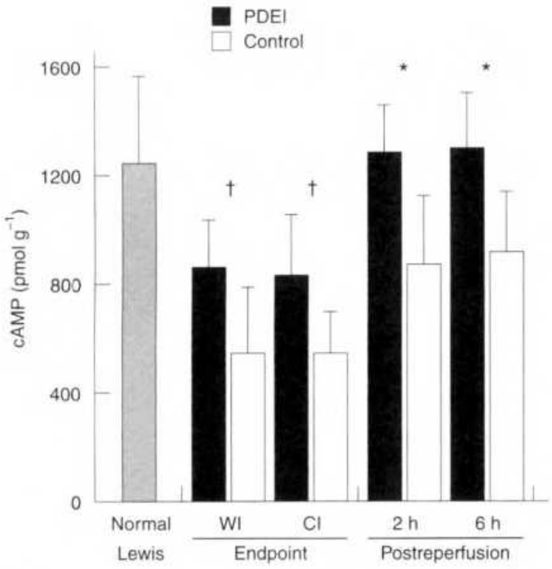


Fig. 3 Changes in hepatic cyclic 3′,5′-adenosine monophosphate (cAMP) concentration. PDEI, phosphodiesterase inhibitor; WI, warm ischaemia; CI, cold ischaemia. $^*P < 0.01$, $^\dagger P < 0.05$ *versus* control (Mann–Whitney *U* test)

Liver tissue cyclic 3′,5′-adenosine monophosphate levels

Changes in hepatic cAMP levels are illustrated in *Fig. 3*. With respect to fresh livers in normal rats, there was a decline in the tissue cAMP in both groups during 60 min of warm ischaemia *in situ* to approximately 40–70 per cent of the initial values. cAMP levels at the end of warm and cold ischaemia were significantly higher in the PDEI group. Subsequent 45 min of storage in cold lactated Ringer's solution did not reduce the tissue cAMP level in either group. Levels in the PDEI group 2 and 6 h after reperfusion were significantly higher than those of the control group, and as high as the concentration in heart-beating normal animals.

Discussion

The main obstacle to use of grafts from non-heart-beating donors is the warm ischaemia that occurs as a result of cardiac arrest before cold preservation. Livers can tolerate simple warm ischaemia for 60 min or more in non-transplant settings[14]. However, in transplant settings they can tolerate only 15–30 min of warm ischaemia because the warm ischaemic insult is potentiated by subsequent cold ischaemia and rewarming[3–5]. In this experiment, 60 min of warm ischaemia was applied as a non-heart-beating donor model with prolonged warm ischaemia, and the possible beneficial effects of a type 3 PDEI on transplanted graft function were investigated. If PDEI did have beneficial effects on graft viability, it would be clinically applicable for marginal liver graft support as well as cardiac support in non-heart-beating donors.

Cellular cAMP, an intracellular second messenger, plays an important role in cellular responses to various stimuli, including ischaemia–reperfusion injury[7–9]. Intracellular cAMP concentrations increase in response to the activation of adenylate cyclase, and decrease in response to the activation of phosphodiesterase[7–9]. Type 3 phosphodiesterase is a physiologically important component in the heart, platelets, vascular and airway smooth muscle, liver, lung, kidney and mononuclear inflammatory cells[7–9].

cAMP levels in the hepatic grafts were significantly improved in the PDEI group at the endpoint of both warm and cold ischaemia, and after reperfusion. The beneficial effects of PDEI could be attributed to several mechanisms: protection of hepatic sinusoidal endothelial cells, relaxation of hepatic stellate cells, inhibition of platelet aggregation and anti-inflammatory effects[15–20]. The sinusoidal endothelial cell lining is the primary target of ischaemia–reperfusion injury[21,22]. Increased serum levels of hyaluronic acid, which is degraded primarily by sinusoidal endothelial cells, indicates dysfunction of those cells after reperfusion of the graft[20]. cAMP has been reported to be an important factor in the maintenance of cell shape and barrier function of sinusoidal endothelial cells in the grafts[8,9,20–22]. The administration of PDEI and the resultant increase in tissue cAMP concentration, not only during warm and cold preservation but also after reperfusion, might have kept sinusoidal endothelial cells intact in the liver graft, in the present study.

The relaxation of hepatic stellate cells and the prevention of platelet activation might have contributed to the improvement of sinusoidal circulation and the subsequent increase in bile production by the graft[15,16]. Kawada and Inoue[15] reported that the relaxation of hepatic stellate cells, which are located in Disse's space and determine sinusoidal tonus, is induced via a cAMP-dependent mechanism. Both vascular smooth muscle and hepatic stellate cells have myosin fibres that contract through the calcium–calmodulin-dependent pathway. Intracellular cAMP inhibits the pathway and relaxes those cells[7–9,15]. cAMP might therefore contribute to the improvement in hepatic microcirculation via this mechanism.

Moreover, concerning the antithrombotic effects of PDEI, Sill *et al.*[16] reported that type 3 PDEIs inhibit platelet activation *in vivo* and *in vitro* through the inhibition of calcium mobilization and glycoprotein IIb/IIIa expression. After warm ischaemia, preserved cAMP levels in the PDEI group might enable efficient flushout of the blood component from hepatic sinusoids by the prevention of sinusoidal endothelial cell injury, relaxation of stellate cells and inhibition of platelet aggregation. Efficient perfusion and procurement of the liver as a result of the effects of PDEI might contribute to the prompt recovery of tissue cAMP levels after reperfusion.

It has also been reported that increases in intracellular cAMP concentration are associated with the prevention of inflammatory reactions including hepatic ischaemia–reperfusion injury[7–9]. The inhibitory effect of type 3 PDEI on lipopolysaccharide has been reported to inhibit the production of tumour necrosis factor production *in vivo* and *in vitro*[19,20]. Taguchi *et al.*[19] recently reported that PDEI inhibits the production of tumour necrosis factor α in activated macrophage/Kupffer cells using the lipopolysaccharide-induced murine hepatitis model. Pober *et al.*[20] demonstrated that an increase in cAMP levels in endothelial cells inhibits the synthesis and expression of E-selectin and vascular cell adhesion molecule 1 in response to tumour necrosis factor.

There appear be two possible explanations for the increased tissue levels of cAMP in the PDEI group during both warm and cold ischaemia and after reperfusion. One explanation is the direct effect of milrinone. The terminal half-life of milrinone by infusion is 2·7 h[8], and it could work not only during the 60 min of warm ischaemia and 45 min of cold ischaemia, but also after reperfusion. The drug activity could therefore have continued even after reperfusion. The other possible explanation is a secondary increase in cAMP concentration in PDEI-treated grafts after reperfusion. Better preservation of the hepatic graft in the PDEI group than in the control group might have resulted in a higher production of cAMP after reperfusion.

In conclusion, milrinone, a type 3 PDEI, attenuated the graft injury caused by warm and cold ischaemia and subsequent reperfusion via an increase in intracellular cAMP levels. The treatment may be a novel pharmacological intervention for safe and efficient usage of liver grafts from non-heart-beating donors.

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Original article

Potential of laparoscopic ultrasonography as an alternative to operative cholangiography in the detection of bile duct stones

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Background: Intraoperative cholangiography (IOC) is time consuming, requires radiation and sometimes fails. In contrast, laparoscopic ultrasonography (LUS) is a comparatively quick, safe and non-invasive technique. The aim of this study was to assess the potential of LUS as an alternative to IOC.

Methods: LUS was performed on 367 patients undergoing laparoscopic cholecystectomy. Laparoscopic duct exploration was performed in the presence of duct stones. Data were collected prospectively. The presence or absence and number of duct stones detected by LUS were recorded. The maximum bile duct diameter determined by LUS was compared with a preoperative ultrasonographic measurement according to age and the presence of duct stones. The final arbiter was the demonstration of stones removed at laparoscopic duct exploration (59 patients) or subsequently by endoscopic retrograde cholangiopancreatography (two patients).

Results: LUS visualized the CBD in 99 per cent of patients and the common hepatic duct in 92 per cent. It identified stones in 56 of the 61 patients with duct stones. No stones were demonstrated in the remaining 306 patients (sensitivity 92 per cent, specificity 100 per cent, positive predictive value 100 per cent, negative predictive value 98 per cent). LUS underestimated the total number of stones in 18 per cent of patients with common duct stones. The mean common bile duct diameter was 5·0 mm before operation and 5·9 mm during the procedure in patients without duct stones, rising significantly to a mean of 9·2 mm before operation and 11·2 mm at LUS in those with duct stones ($P < 0{\cdot}0001$).

Conclusion: The combination of the demonstration of duct stones and bile duct diameter measurement makes LUS a potential replacement for IOC. Improved demonstration of the common hepatic duct would be advantageous.

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Introduction

Evaluation of the common bile duct (CBD) during cholecystectomy is undertaken for two reasons. The first is to determine the presence or absence of bile duct stones. Second, it is increasingly important to demonstrate the biliary anatomy at operation to help avoid or recognize bile duct injury. Intraoperative cholangiography (IOC) is at present generally regarded as the 'gold standard' for this purpose. The technique itself has several disadvantages. It is time consuming, adding an extra 13–18 min to the procedure time[1–4]; requires exposure of the patient, operator and theatre personnel to radiation; and has a failure rate of 3–17 per cent[2,4–7]. In contrast, laparoscopic ultrasonography (LUS) is a safe, non-invasive and more rapid technique, adding only 5–10 min to the procedure time[2,4,8–10]. Previous studies have shown LUS to be feasible in 93–100 per cent of cases[2,4,7,11,12]. These studies demonstrated a sensitivity of 80–100 per cent and a specificity of 98–100 per cent, compared with IOC, with respective values of 64–80 and 97–100 per cent in the detection and exclusion of bile duct stones[2–4,8]. However, only 4–9 per cent of patients in these published series had CBD stones (range 2–54 patients).

The aim of this study was to assess the ability of LUS successfully to identify and accurately to determine biliary anatomy and the presence or absence and number of duct stones in a larger series of 61 patients with CBD stones. Preoperative and peroperative bile duct diameters as a predictor of ductal stones, either alone or in combination with stone visualization, were also evaluated.

Table 1 Patient details according to the presence or absence of duct stones

	No duct stone (*n*=306)	Duct stones (*n*=61)	Total (*n*=367)
Sex ratio (M : F)	72 : 234	19 : 42	91 : 276
Mean age (years)	51	54	52
Colic (%)	62	45	59
Inflammation (%)	7	3	6
Jaundice (%)	8	49	15
Pancreatitis (%)	23	3	20

Patients and methods

From September 1996 to November 1999, 367 consecutive patients (276 women and 91 men; mean age 52 years) undergoing laparoscopic cholecystectomy with or without CBD exploration were incorporated at the time of operation into a database recording the patient's age, sex and presenting symptoms. Liver function tests (estimation of bilirubin, alkaline phosphatase and alanine aminotransferase levels) and an ultrasonographic scan of the bile ducts measuring the maximum CBD diameter were performed less than 1 week before operation and recorded in the database.

At the time of laparoscopy, after dissection of the cystic duct, an intraoperative ultrasonographic scan of the biliary system was performed with a 7·5-MHz Aloka linear laparoscopic ultrasound probe (Keymed, Southend, UK). The first 30 examinations were performed under the supervision of a consultant radiologist or sonographer. All subsequent examinations were undertaken by a consultant surgeon, a trainee under supervision or a trainee alone, once experienced. The patient's history was known at the time of the examination.

The mid portion of the CBD was identified under laparoscopic vision. Saline was instilled into the abdomen in order to achieve improved ultrasound contact. The bile duct was identified by ultrasonography with reference to the portal vein and hepatic artery. It was then followed up into the liver and down through the pancreas to the ampulla. The ability to recognize the common hepatic and common bile ducts was noted. The maximum CBD diameter (in millimetres) was measured. Filling defects casting acoustic shadows were noted, and the number and size of the largest stones were recorded.

IOC was undertaken after LUS only in patients at high risk of ductal stones defined by abnormal liver function test results or a dilated CBD in the week before operation[13]. IOC was performed with an image intensifier. Sufficient volume of contrast was used to outline the biliary tree. If ductal stones were seen on LUS or IOC, a laparoscopic bile duct exploration was performed. The number and size of suspected stones demonstrated by LUS was compared with the presence and number of stones removed at the time of duct exploration (59 patients) or subsequent endoscopic retrograde cholangiopancreatography (ERCP) (two patients with missed stones). The mean length of follow-up was 24·8 (range 6–56) months.

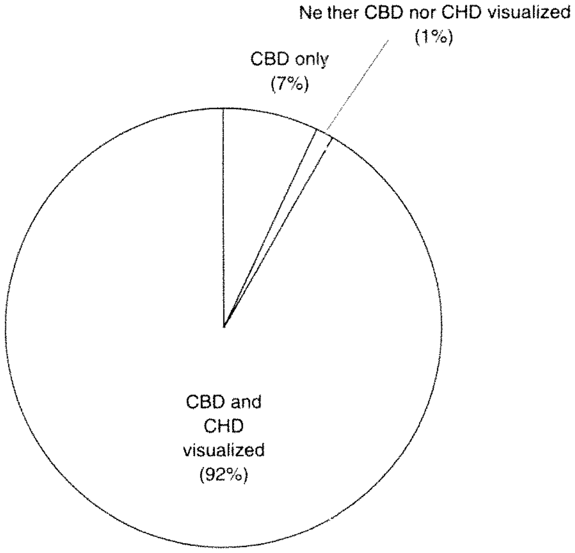

Fig. 1 Laparoscopic ultrasonographic duct visualization. CBD, common bile duct; CHD, common hepatic duct

Results

The clinical presentation of the 367 patients in the study is detailed in *Table 1*. Adequate intraoperative ultrasonographic visualization of the CBD occurred in 362 (99 per cent) of 367 patients and of the common hepatic duct in 336 (92 per cent) of 367 patients. The overall failure rate of CBD and common hepatic duct visualization was therefore 1 and 8 per cent respectively (*Fig. 1*).

LUS correctly identified 56 of the total number of 61 patients with duct stones. Of the five cases where LUS failed to identify the stones, two patients had one 3-mm stone identified in each case by IOC; the stones were flushed through the ampulla. A third patient had two stones of 10 mm in maximum diameter (previously demonstrated by ERCP and identified by IOC), which were removed via a choledochotomy. Two patients presented later with missed stones. No stones were demonstrated in the remaining 306 patients undergoing LUS, and these patients have not subsequently reported symptoms suggesting retained stones at a mean follow-up of 24·8 months. Thus, in terms of the detection and exclusion of bile duct stones, the

Table 2 Common bile duct diameter in patients with and without duct stones measured by preoperative ultrasonography and laparoscopic ultrasonography

	CBD diameter (mm)
No duct stones	
Preop. ultrasonography	5·0(3·9)
LUS	5·9(4·4)
CBD stones present	
Preop. ultrasonography	9·2(7·0)*
LUS	11·2(6·9)*

Values are mean(2s.d.). CBD, common bile duct; LUS, laparoscopic ultrasonography. *$P < 0·001$ *versus* patients with no duct stone (*t* test)

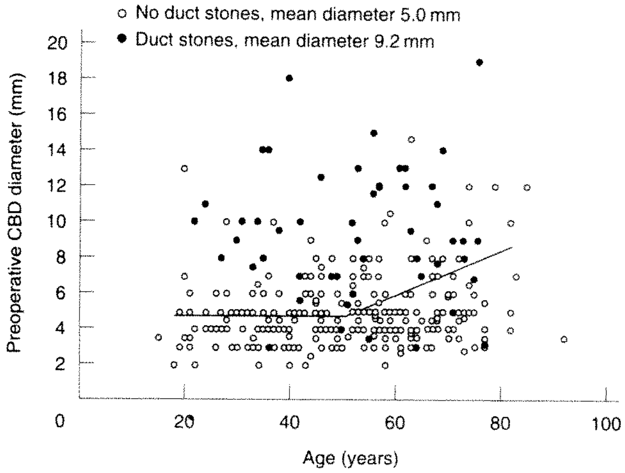

Fig. 2 Preoperative diameter of the common bile duct (CBD) determined by ultrasonography according to age in patients with and without bile duct stones. The solid line represents previously quoted normal values[13]

sensitivity and specificity of LUS were 92 and 100 per cent respectively, with positive and negative predictive values of 100 and 98 per cent respectively. When estimating the number of stones in the duct, intraoperative ultrasonography underestimated the total number of stones in ten patients (18 per cent) by a median of 1 stone.

The mean CBD diameter was 5·0 mm before operation and 5·9 mm during the procedure in patients without duct stones, rising significantly to a mean of 9·2 mm before surgery and 11·2 mm at LUS in those with CBD stones ($P < 0·0001$), as shown in *Table 2*. *Figs 2* and *3* show the individual values of CBD diameter according to age in patients with and without duct stones undergoing preoperative and laparoscopic ultrasonography. The line drawn on the graphs denotes a previously described normal CBD diameter measured by preoperative ultrasonography dependent on the patient's age[13]. In the present study, however, seven patients with duct stones had a CBD diameter measured by preoperative ultrasonography falling on or below this line (*Fig. 2*), whereas the line represented a consistent predictor for the absence of duct stones in patients undergoing LUS (*Fig. 3*).

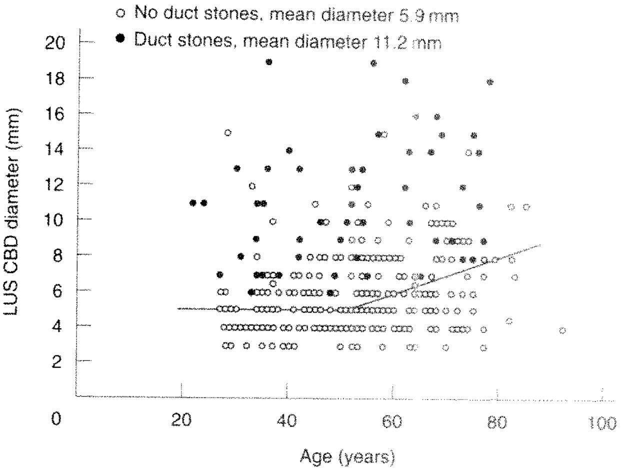

Fig. 3 Diameter of the common bile duct (CBD) determined by laparoscopic ultrasonography (LUS) according to age in patients with and without bile duct stones. The line represents previously quoted normal values[13]

IOC was attempted in addition to LUS in 126 patients at high risk of CBD stones, determined by the preoperative assessment. IOC failed in seven patients (6 per cent) and revealed duct stones in 57 of the 61 patients, although there were two false-positive and four false-negative cholangiograms (two patients with stones missed by cholangiography alone, and two missed by both IOC and LUS). The sensitivity and specificity of IOC in the detection of duct stones was therefore 93 and 97 per cent respectively.

The two patients with missed stones were not identified by either LUS or IOC at the time of operation. One patient presented 3 months subsequently with jaundice and the second presented 3 weeks after operation with biliary colic. Both patients underwent a successful ERCP and sphincterotomy. The results of LUS and IOC are summarized in *Fig. 4*.

Discussion

The aim of this study was to determine whether LUS has the potential to replace IOC as the main imaging modality during laparoscopic cholecystectomy, the purpose of which is to detect or exclude duct stones and to demonstrate biliary anatomy. It is therefore important to show that LUS is comparable or superior to IOC in these two aspects. This

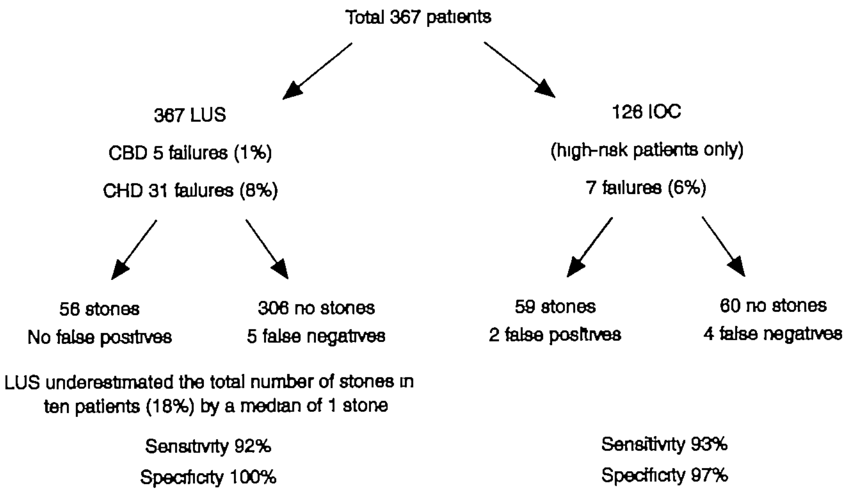

Fig. 4 Flow diagram of results. CBD, common bile duct; CHD, common hepatic duct; IOC, intraoperative cholangiography; LUS, laparoscopic ultrasonography

study has the advantage of including a large number of patients with duct stones (61 patients; 17 per cent) compared with other smaller series. In addition the long mean follow-up period of 24·8 months allowed for the re-presentation of patients with missed stones.

The ability of LUS to detect bile duct stones was examined and found to have a sensitivity and specificity of 92 and 100 per cent respectively, which is similar to previous reports (80–100 and 98–100 per cent respectively)[2–4,8,14]. This compares favourably with the calculated sensitivity and specificity of 93 and 97 per cent for IOC in the present study (based on a policy of selective cholangiography). Other studies of IOC quote a sensitivity of 64–80 per cent and a specificity of 97–100 per cent for the detection of CBD stones[2–4,8]. It has been suggested that LUS is more sensitive than IOC at identifying small stones and sludge[1,2]; however, in two patients LUS failed to identify a single 3-mm stone subsequently detected by IOC. These stones were easily flushed through the ampulla. Although it is more important to detect or exclude duct stones than to determine the exact number of stones in the duct, this information is helpful during duct exploration. LUS underestimated the total number of stones in ten patients (18 per cent) by a median of 1 stone; this may be due to the ultrasound echo from one stone obscuring another.

The CBD diameter measured by LUS was a good negative predictor of ductal stones (*Fig. 4*), whereas the common duct diameter measured by preoperative ultrasonography was not as consistent a negative predictor. There was a complete absence of bile duct stones in ducts of 5 mm or less in diameter at age 50 years, then increasing by 1 mm for each decade to 8 mm at 80 years of age. In these circumstances, LUS is an absolute negative predictor for bile duct stones.

The second important question is whether LUS can adequately demonstrate biliary anatomy compared with IOC. The overall failure rate of CBD visualization by LUS was 1 per cent in the present study, but adequate demonstration of the common hepatic duct was more difficult, failing in a further 7 per cent of cases. These results are comparable to the failure rate of 6 per cent for IOC. These failure rates of LUS and IOC are similar to those of other published reports, of 0–7 and 3–17 per cent respectively[2,4–7,11,12].

Interpretation of the ductal system at LUS is usually straightforward and the ducts can be followed from the cystic duct and common hepatic duct to the common duct junction with the duodenum. Barteau *et al.*[15] expressed concern at the impaired ability of LUS to recognize the intrapancreatic CBD. John *et al.*[16], using a 90° sector scanner, were unable to visualize the intrapancreatic CBD and pancreatic duct in 20 and 15 per cent of cases respectively, whereas the suprapancreatic common duct was visualized in 100 per cent of cases. The present authors have not found visualization of the intrapancreatic CBD difficult. Of greater concern is the 8 per cent failure rate in demonstrating the common hepatic duct. The advent of superior ultrasound probes with the added facility of duplex scanning should improve the identification of the whole biliary tree and its variations. A further advantage of LUS is the benefit of specific anatomical information during the difficult dissection of an inflamed Calot triangle[12]. In addition, LUS can diagnose clinically relevant incidental abnormalities at the time of operation[5].

In summary, intraoperative ultrasonography is non-invasive and does not expose patients and staff to radiation. LUS is more specific than IOC for the detection of ductal stones, with a comparable sensitivity and failure rate. LUS, therefore, may have the potential to replace IOC for both the determination of biliary anatomy and the detection of CBD stones. Blinded prospective studies are required for confirmation. As technology advances and the quality of ultrasonographic scanners improves, LUS may eventually become a superior technique.

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Use of cadaveric superior mesenteric artery as interpositional vascular graft in orthotopic liver transplantation

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Background: Hepatic artery thrombosis remains the most common technical complication that causes graft failure following orthotopic liver transplantation. The development of split liver and living related liver transplantation has led to the use of shorter and smaller arteries for arterial reconstruction to the graft. The present aim was to assess the effectiveness of the superior mesenteric artery as an interpositional graft in arterial reconstruction during liver transplantation.

Methods: Cadaveric superior mesenteric artery was used to reconstruct small and short or multiple hepatic arteries in 35 liver transplants including 29 split, three living related, two whole liver transplants and one emergency revascularization post-transplantation.

Results and conclusion: A low incidence of hepatic artery thrombosis (one of 35 patients) was achieved utilizing cadaveric superior mesenteric artery as an interpositional vascular graft in liver transplantation.

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Introduction

Arterial reconstruction during orthotopic liver transplantation is an important step in the success of the procedure[1,2]. The reconstruction is dependent on the anatomy and size of the donor and recipient hepatic arteries. Recognition of anatomical variants and knowledge of appropriate techniques of vascular anastomosis are important[3].

The authors have modified the 120° triangulating technique used by microvascular surgeons[4] and have used it routinely for both adult and paediatric liver transplants. Arterial conduits using donor iliac arteries were developed to overcome arterial inflow problems. When the main problems are length, size discrepancy or reconstruction of more than one artery, the use of donor interpositional vascular grafts is more appropriate. A wide range of grafts has been used, including internal or external iliac artery, splenic artery, reversed saphenous vein and superior mesenteric artery (SMA). The SMA trunk is a particularly useful interpositional graft for overcoming problems related to multiple and small-diameter donor arteries, with a low incidence of hepatic artery thrombosis. The expansion of split liver and living donation programmes suggests that there may be an increasing role for the use of this graft.

Patients and methods

Some 1625 consecutive liver transplants performed between January 1989 and September 1999 for acute or chronic liver disease in adults and children were reviewed. Rearterialization of the liver was achieved mostly by means of a previously described 120° triangulating technique using interrupted 6/0 or 7/0 polypropylene sutures.

The SMA in an adult is approximately 25 cm in length and, even if an accessory right hepatic artery is present or if retrieval of the pancreas is planned, a 10-cm segment can still be harvested at the end of multiorgan retrievals. In this centre the SMA is routinely retrieved if the liver is potentially divisible or if a living related procedure is planned for the same week. The cadaveric vessels are stored in University of Wisconsin preservation solution at 4°C for no longer than 10 days to avoid damage to the media. Only ABO-compatible cadaveric vessels have been used for vascular reconstruction. The diameter of an adult SMA at origin ranges from 6 to 10 mm and is adequate for anastomosis to the recipient's common hepatic artery or to an iliac artery conduit. The reconstruction and lengthening of a right or left hepatic artery is usually performed on the back table using the main distal stump of the SMA or its

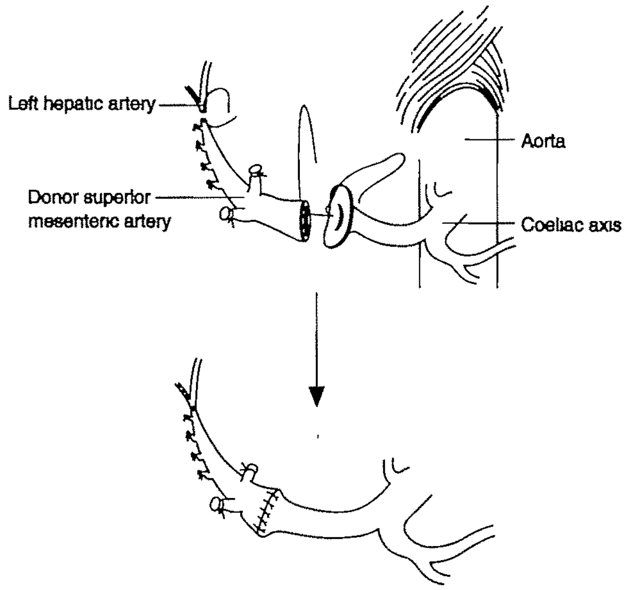

Fig. 1 Use of superior mesenteric artery as interpositional vascular graft in liver transplantation

major branches, usually a jejunal or ileal artery, with interrupted 7/0 or 8/0 polypropylene sutures (*Fig. 1*).

Results

The technique has been used in 35 liver transplants carried out in the authors' unit. Of these, nine were children with a mean age of 3·8 (range 0·5–16) years and mean weight of 15 (range 5–54) kg. The remaining 26 patients were adults of mean age 46·3 (range 18–68) years. All were patients requiring reconstruction of small and short or multiple arteries, including 29 split liver transplants (27 right lobes, two left lateral segments) and three living related transplants (two left lateral segments, one left lobe graft). The remaining three cases were two whole liver grafts and one emergency revascularization 3 days after liver transplantation for hepatic artery thrombosis.

At a mean follow-up of 29 months there has been one hepatic artery thrombosis in this group of 35 patients at high risk for vascular complications. The hepatic artery thrombosis occurred in a child who developed collaterals rapidly, but has not required retransplantation.

Discussion

The technique of reduced-size segmental liver grafts has been extended to the development of split liver and living related liver transplantation. The implantation of such segmental grafts may be complicated by the presence of short vessels of small calibre[5].

In split liver transplantation the main trunk of the hepatic artery and portal vein is allocated to the left or right lobe depending on the preferences of the centre performing the split procedure. This leaves one lobe with short, small and sometimes multiple vessels to be reconstructed later on the back table or in the recipient. Similar problems are encountered in living related liver transplantation when short or small or multiple hepatic arteries may need to be reconstructed and lengthened. Use of split or living related left lateral segments or left lobe grafts increases the incidence of double or triple arterial supply to 10–60 per cent[6].

A variety of techniques for lengthening the donor artery have been used including the use of the infrarenal aortic donor iliac arterial conduits[7] and interpositional grafts including internal and external iliac arteries, splenic artery, reversed saphenous vein and SMA. If cadaveric vessels are not available, vascular grafts may include the inferior mesenteric vein, sigmoid or inferior epigastric arteries of the recipient[8,9].

Cadaveric arteries stored for up to 14 days in Terasaki solution (lymphocyte culture medium) have been used safely in paediatric liver transplantation without increasing the rate of hepatic artery thrombosis when compared with fresh arteries. The same authors compared University of Wisconsin with Terasaki solution as a storage fluid for vascular homografts and found no difference in the histology after a 14-day storage period[10].

The SMA has proved particularly useful and versatile because of its conical shape, which allows it to be used to overcome large size discrepancies, thereby gradually reducing abnormalities of blood flow secondary to the sharp modification in the calibre of the lumen. The presence of multiple branches has also proved ideal in reconstructing multiple arteries into a single vessel.

The SMA is a valuable interpositional graft mainly for difficult vascular reconstructions where both length and size discrepancy may jeopardize the success of the arterial anastomosis; it appears to be associated with a low incidence of hepatic artery thrombosis. With the expansion in split liver and living related liver transplant programmes, there will be an increasing need for a versatile interpositional graft as described in this report.

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Original article

Postoperative liver function after elective right hepatectomy in elderly patients

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Background: Because the influence of age on the risk of liver resection is controversial, the outcome of major liver resection in patients older than 65 years was evaluated.
Methods: Between 1990 and 1997, 24 patients aged 65 years or more underwent elective right hepatectomy (segments V–VIII) for a malignant liver tumour arising in a normal liver. They were evaluated retrospectively in terms of operative deaths, morbidity and postoperative liver function, and compared with 22 patients aged 40 years or less who had undergone a similar resection during the same interval.
Results: Elective right hepatectomy in patients aged 65 years or more resulted in a similar number of deaths (one *versus* none) and a similar severe complication rate (12 *versus* 5 per cent) to that observed in patients aged 40 years or less. Evaluation of liver function on days 2, 5 and 8 after operation, in patients aged 65 years or more and 40 years or less, showed that mean prothrombin time was 52 *versus* 56 per cent, 58 *versus* 62 per cent and 76 *versus* 72 per cent respectively (*P* not significant) and that the mean total serum bilirubin level was 35 *versus* 38 µmol/l, 32 *versus* 36 µmol/l and 25 *versus* 28 µmol/l (*P* not significant). Postoperative levels of aminotransferases, γ-glutamyl transpeptidase and alkaline phosphatase were similar in the two groups. Mean(s.d.) duration of hospital stay was 15(7) days in patients aged 65 years or more and 13(4) days in the younger patients.
Conclusion: Postoperative liver function after elective right hepatectomy in selected patients older than 65 years was similar to that in younger patients.

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Introduction

Because the average age of the population is increasing, a number of studies have investigated the risk of liver resection in elderly patients with some[1–3] but not all[4–6] showing an increased mortality rate compared with that in younger patients. However, some of these studies had a long period of observation, and included patients with a varying extent of resection in normal and abnormal underlying liver parenchyma[1,3,6]. There are several possible reasons for an increased risk in elderly patients. Such patients are more likely to have associated medical conditions, such as diabetes or coronary artery disease. Furthermore, liver regeneration may be impaired owing to a reduced blood flow and responsiveness to potentially hepatotrophic hormones[7]. A high proportion of elderly patients with potentially resectable liver tumours may therefore not be referred for surgery. The policy in this unit over the past 10 years has been to rely mainly on the American Society of Anesthesiologists (ASA) physical status score and not to use age alone as a contraindication to hepatic resection[8].

To evaluate tolerance to major hepatic resection in patients aged more than 65 years, a retrospective study was undertaken to compare two groups of patients with a clear age difference who underwent the same major resection (segments V–VIII) with normal underlying liver parenchyma.

Patients and methods

Data on all patients who had had a right hepatectomy (removing Couinaud's segments V–VIII) between 1990 and 1997 were retrieved from an ongoing database. Only those who fulfilled the following criteria were selected for the present study: (1) elective surgery for a malignancy, (2) no associated extrahepatic surgery, (3) surgery performed through an abdominal incision without opening of the diaphragm, (4) no underlying liver disease (in particular neither fibrosis nor steatosis), and (5) ASA grade I and II. Of these, 24 patients were aged 65 years or more (mean 70 (range 65–80) years). They were compared with a group of 22 patients aged 40 years or less (mean 32 (range 17–40) years) who fulfilled the same criteria. During that interval, 17 other patients older than 65 years underwent a right hepatectomy but were excluded as they did not fulfil the study criteria.

Patients aged 65 years or more and 40 years or less were comparable with respect to preoperative liver function, ASA grade, rate and duration of pedicular clamping, and weight of the resected specimen (*Table 1*). In addition, the technique of right hepatectomy was standardized during the study period. In brief, the right liver was fully mobilized, the hepatocaval ligament was transected between sutures, and the main trunk of the right hepatic vein was controlled extraparenchymally. The right hepatic artery and the right branch of the portal vein were dissected and transection-ligated in the hilus. Parenchymal transection was performed along the main portal fissure using an ultrasonic dissector (Dissectron™; Satelec Medical, Mérignac, France). Haemostasis and biliostasis were obtained with bipolar electrocautery except for the large structures, which were ligated on the side of the non-resected segments and clipped on the side of the specimen. After parenchymal transection, the hepatic vein was ligated as well as the right biliary duct.

Prothrombin time (PT; expressed as a percentage of normal), serum aspartate aminotransferase, alanine aminotransferase, γ-glutamyl transpeptidase (GGT), alkaline phosphatase and bilirubin serum level were measured before surgery and on days 2, 5 and 8 after operation.

Comparison between variables was performed using Fisher's exact test or the median *t* test as required.

Results

There was one in-hospital death, from a myocardial infarction on day 1 after operation, in a 65-year-old patient with no previous history of cardiac disease, a normal preoperative electrocardiogram and who had been classified as ASA I. The incidence of severe postoperative complications was not significantly different between the two groups (three (12 per cent) of 24 *versus* one (5 per cent) of 22) (*Table 2*). After exclusion of the patient who had a myocardial infarction, of the three patients who experienced severe postoperative complications, two patients, aged more than 65 years, required percutaneous drainage of a postoperative abdominal collection and one patient, aged less than 40 years, was reoperated on postoperative day 2 for bleeding from the cut surface. The most frequent minor complications were pleural effusion and atelectasis (*Table 2*).

Table 1 Characteristic of patients aged 65 years or more and 40 years or less undergoing elective right hepatectomy

	Age ≥ 65 years (*n*=24)	Age ≤ 40 years (*n*=22)
Sex ratio (M : F)	11 : 13	16 : 6
ASA grade		
I	10	14
II	14	8
Indications		
Primary tumour	3	17
Metastasis	21	5
Preoperative liver function tests*		
AST (units/l)	23(11)	29(20)
Prothrombin time (% of normal)	93(10)	92(10)
Bilirubin (μmol/l)	10(4)	12(4)
Operative variables		
Continuous pedicular clamping	12	14
Intermittent pedicular clamping	12	8
Duration of pedicular clamping (min)*	43(14)	38(9)
Operative time (min)*	287(85)	263(92)
Weight of resected specimen (g)*	1080(1230)	1213(1348)

*Values are mean (s.d.). ASA, American Society of Anesthesiologists; AST, aspartate aminotransferase

Table 2 Postoperative complications in patients aged 65 years or more and 40 years or less after elective right hepatectomy.

	Age ≥ 65 years (*n*=24)	Age ≤ 40 years (*n*=22)
Severe complications		
Myocardial infarction	1	0
Postoperative bleeding	0	1
Subphrenic collection	2	0
Other complications		
Pleural effusion	7	6
Atelectasis	4	4

Some patients had more than one complication

Overall, 14 patients required intraoperative or postoperative transfusion. This incidence was comparable in the two groups (eight *versus* six patients; *P* not significant) and the number of packed red blood cells transfused was also comparable (mean(s.d.) 1·3(2·5) *versus* 1·1(2·3) units). Neither whole blood nor fresh frozen plasma was transfused.

Postoperative kinetics of liver function tests are summarized in *Figs 1* and *2*. The most important changes occurred by day 2 after operation and consisted of a decrease in PT and an increase in serum bilirubin and transaminase levels. These variations were comparable in the two groups and the

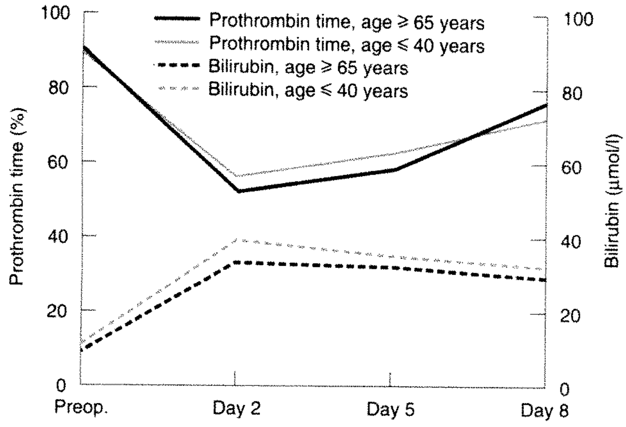

Fig. 1 Prothrombin time and serum bilirubin level after elective right hepatectomy in patients aged 65 years or more and 40 years or less

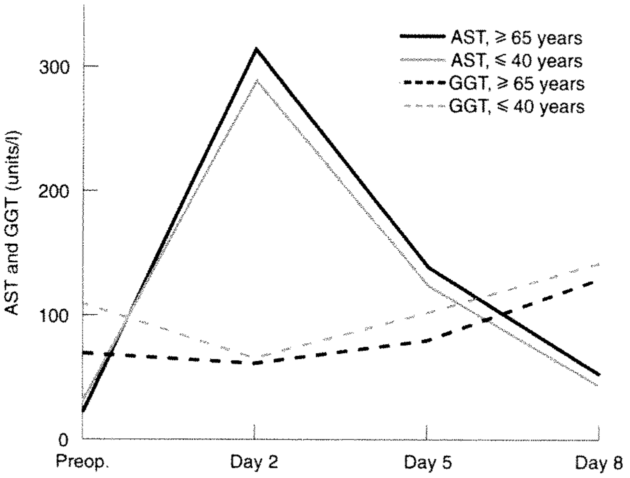

Fig. 2 Serum levels of aspartate aminotransferase (AST) and γ-glutamyl transpeptidase (GGT) after right hepatectomy in patients aged 65 years or more and 40 years or less

tests normalized at the same rate. The kinetics of GGT were comparable in the two groups. The serum level of alkaline phosphatase paralleled that of GGT and did not differ significantly between the two groups of patients on days 2, 5 and 8 after operation: 94 *versus* 103 units/l, 146 *versus* 139 units/l and 174 *versus* 163 units/l respectively (*P* not significant).

The mean(s.d.) duration of hospital stay was 15(7) (range 6–22) days in patients aged 65 years or more and 13(4) (range 8–24) days in the younger patients (*P* not significant).

Discussion

Previous studies that have investigating the influence of age on the short-term outcome following hepatectomy have yielded conflicting results (*Table 3*). The results of these studies are difficult to interpret for several reasons: (1) criteria by which the decision to operate on an elderly patient was made were not clarified; (2) most studies included minor procedures, for which the influence of ageing on liver tolerance to resection is unlikely to be significant; (3) comparison was performed between patients above or below a certain age but the age ranges in each group were not given and it is therefore unclear to what extent the two groups differed; and (4) the two groups frequently had different liver-associated risk factors such as the presence or severity of an underlying liver disease or extent of resection. Also these studies focused on in-hospital mortality and morbidity but not on the biological tolerance of the parenchyma to surgery.

The approach used in the present study aimed at avoiding these potential biases, and relied on the ASA score rather than age to select for surgery. All patients included had undergone the same major resection of four segments. The control group included patients aged 40 years or less and

Table 3 Influence of age on the risk of liver resection

Reference	Year	No. of patients	Age (years)	Cirrhosis (%)	Resection ≥ 4 segments (%)	Mortality rate (%)*	*P*
Ezaki *et al.*[9]	1987	37	≥ 66	68	13	3 *versus* 5	n.s.
Yanaga *et al.*[1]	1988	27	≥ 65	63	44	41 *versus* 21	< 0·02
Fortner and Lincer[2]	1990	90	≥ 65	—	64	11 *versus* 4	< 0·05
Mentha *et al.*[10]	1992	52	≥ 65	8	31	6 *versus* 3	n.s.
Nagasue *et al.*[3]	1993	32	≥ 70	69	31	12 *versus* 0	n.s.
Zieren *et al.*[4]	1994	18	> 70	—	28	6 *versus* 3	n.s.
Takenaka *et al.*[11]	1994	39	≥ 70	79	0	5 *versus* 1	n.s.
Fong *et al.*[5]	1997	133	≥ 65	6	49	4 *versus* —	n.s.
Wu *et al.*[6]	1999	21	≥ 80	81	14	0 *versus* 2	n.s.
Present study	2000	24	≥ 65	0	100	4 *versus* 0	n.s.

*In elderly compared with younger patients. n.s., Not significant was therefore clearly different from the study group with patients aged 65 years or more. In addition, the postoperative kinetics of liver function tests were compared in an attempt to assess whether the parenchymal damage and regenerative potential following resection were age dependent. It was shown that, following a major resection, the mortality and morbidity rates as well as the nature of complications were comparable in the old and young groups. This result is comparable to that of Fong *et al.*[5] but differs from those of others[1,2,11] who reported an increased risk in older patients. The most frequently reported cause of death in elderly patients with no underlying liver disease undergoing liver resection is hepatic insufficiency[2,5], myocardial infarction[2,4], pneumonia[2,5] and gastrointestinal bleeding[2].

One patient in this study died from myocardial infarction, despite a normal preoperative electrocardiogram and no previous cardiac history, an experience shared by others[2]. Routine non-invasive preoperative coronarographic assessment is now undertaken in older patients (65 years or more). Fluid replacement should also be used with caution in the postoperative period to avoid congestive failure or dysrhythmia, the most frequent non-lethal complications[2]. Pulmonary complications were the most frequent and gastrointestinal bleeding the potentially most difficult complication to predict following liver resection, irrespective of age[12].

The previously reported high incidence of postoperative liver failure in elderly patients undergoing major resection, despite the absence of an associated cirrhosis, is a crucial issue[5]. Indeed, this hazard is the rationale in particular for not considering older patients as potential split-liver donors[13,14]. However, safe use of old cadaveric liver grafts has been reported[15,16], demonstrating that the old liver maintains a good functional reserve with regenerating capacity[17]. Analysis of the postoperative kinetics of liver function tests in the present study supports this finding. The magnitude of the PT decrease and bilirubin increase was comparable to that in the younger age group and time to recovery was also similar. Furthermore, the increase in serum GGT and alkaline phosphatase levels, which can be used as markers of liver regeneration, was not delayed. Extensive hepatic resection should not be therefore be contraindicated in elderly patients provided that no additional injury is imposed to the remnant liver parenchyma.

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Suitability for endovascular aneurysm repair in an unselected population

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Background: Tertiary referral centres report that up to 60 per cent of patients may be suitable for endovascular repair of abdominal aortic aneurysm (EVAR). The aim of this study was to determine the percentage of abdominal aortic aneurysms (AAAs) presenting to a county-wide vascular service that were suitable for EVAR, and to examine the outcome of subsequent AAA repair in relation to aneurysm morphology.

Patients and methods: All patients being assessed for AAA repair between January 1998 and December 1999 underwent spiral computed tomography angiography to determine aneurysm morphology and suitability for EVAR. Subsequent outcome for all patients in the study was recorded in a prospective vascular database.

Results: A total of 115 patients was assessed. Sixty-three aneurysms (55 per cent) had one or more absolute contraindications to EVAR, a further 13 (11 per cent) had at least one relative contraindication, and 39 (34 per cent) had no contraindication. Of patients with no absolute contraindication to EVAR, ten underwent successful EVAR, five did not meet recognized criteria for surgery, one awaits EVAR, four remain under observation, one awaits open repair, and 31 underwent open repair without death.

Conclusion: Only 30 per cent of unselected AAAs presenting to a vascular service are entirely suitable for EVAR; most of these patients can safely undergo open AAA repair. These data suggest that increased use of EVAR is only possible by deploying devices in suboptimal morphology, and in treating patients who would not normally be considered for open AAA repair.

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Introduction

Since Parodi[1] first reported endovascular repair (EVAR) of abdominal aortic aneurysm (AAA), the technique has become increasingly available, with a number of commercially produced devices of various configurations currently in production[2]. While some reports suggest that EVAR may reduce the mortality rate following elective AAA repair[3,4], there remains concern about the complication rates and long-term durability of endovascular prostheses[2,4–7]. Randomized trials of the technique have begun in a limited number of centres.

At the present time it is not entirely clear what percentage of AAAs would be suitable for EVAR with current devices. Before the introduction of aortouni-iliac devices, reports indicated that between 15 and 47 per cent of AAAs were suitable for EVAR[3,8–10]. The development of the aortouni-iliac graft, combined with a femoro-femoral crossover graft, was said to offer the flexibility that enabled the majority of AAAs to be treated by EVAR[11]. However, these reports have all originated from tertiary units whose referral populations may not be representative of the AAA population encountered in the majority of vascular practices. This study was undertaken to determine the percentage of AAAs, assessed by a single vascular service covering an entire county, that were morphologically suitable for EVAR and to examine the outcome in all such patients.

Patients and methods

The Vascular Unit of the Royal Cornwall Hospital (RCHT) is the sole provider of vascular services in the county of Cornwall (population 480 000). Whilst approximately 80 000 of this population live in an area also served by adjacent District General Hospitals, the remaining 400 000 are served exclusively by the RCHT. All vascular referrals made within the county (both National Health Service and private) are seen and assessed by one of two consultant vascular surgeons. Since late 1997, all non-ruptured aortic aneurysms being considered for either open or endovascular surgical repair have undergone assessment according to the protocol outlined in *Fig. 1*. While all patients undergo computed tomography (CT) angiography to determine morphological suitability for EVAR, it is currently policy in this unit to undertake further detailed assessment only in those patients whose co-morbidity places them at high risk from open AAA repair.

CT angiography is carried out on a Siemens Somatom Plus 4 Scanner (Siemens Medical, Oldbury, UK), using spiral acquisition reconstructed at 5- or 7·5-mm intervals. Multiplanar reformatting is also used and films are then reviewed at a weekly meeting between consultant vascular surgeons and radiologists. Following a detailed preoperative assessment (including formal assessment of pulmonary and cardiac function in selected cases), patients in whom no adverse risk factors are identified are offered open surgical repair; current local audited figures indicate that this has a 7 per cent mortality risk. Patients assessed to be at increased risk from open repair and whose CT suggests that they may be suitable for EVAR, undergo further assessment with either calibrated angiography, or 3-dimensional CT reconstruction, to confirm their suitability for EVAR.

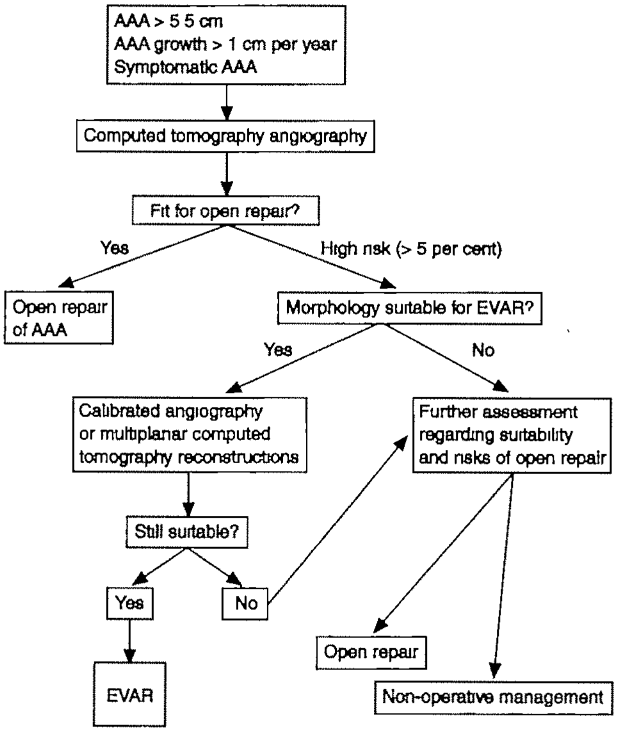
AAA > 5·5 cm
AAA growth > 1 cm per year
Symptomatic AAA
Computed tomography angiography
Fit for open repair?
Yes
High risk (> 5 per cent)
Open repair of AAA
Morphology suitable for EVAR?
Yes
No
Calibrated angiography or multiplanar computed tomography reconstructions
Further assessment regarding suitability and risks of open repair
Still suitable?
Yes
No
EVAR
Open repair
Non-operative management

Fig. 1 Protocol for assessment of abdominal aortic aneurysms (AAA) being considered for surgical repair. EVAR, endovascular repair

Results

During the 2-year interval ending 31 December 1999, a total of 115 patients (mean age 71 (range 57–85) years, 94 men, 21 women) with AAAs, who were considered fit for an endovascular procedure, was assessed for elective repair. Some 114 underwent CT angiography and one patient had aortoiliac angiography at the time of coronary angiography that indicated morphology unsuitable for EVAR; this patient did not have preoperative CT angiography.

Morphological assessment

Sixty-three aneurysms (55 per cent) had one or more morphological features considered to be an absolute contraindication to EVAR with currently available devices. The commonest contraindication was inadequate length of normal infrarenal aorta to enable safe proximal deployment (*Table 1*).

The remaining 52 AAAs had no absolute contraindication to EVAR; however, 13 had morphological features that were relative contraindications (*Table 2*). Only 39 aneurysms were suitable for EVAR on the basis of CT angiography alone. Five of these did not meet generally accepted indications for surgical repair, being asymptomatic or less than 5 cm in maximum anteroposterior diameter, leaving only 34 (30 per cent) that were entirely appropriate for EVAR.

A bifurcated device could be deployed in up to 38 (73 per cent) of the aneurysms appropriate for EVAR. To achieve this figure, however, modular devices enabling differential limb lengths (four patients) and permanent occlusion of both internal iliac arteries (eight patients) would have been required to deploy a bifurcated device. In 14 aneurysms the aortouni-iliac device offered the only practical configuration likely to achieve total exclusion of the AAA.

Outcome

In the 47 patients with no absolute contraindication to EVAR and an appropriate indication for surgical intervention, ten underwent successful EVAR (four had aortouni-iliac devices and six had bifurcated devices), one patient awaits EVAR, one patient awaits open repair, and 31 patients have undergone open repair. The remaining four patients were deemed inappropriate for AAA surgery on the basis of co-morbidity resulting in excessive operative risk (two patients), and patient refusal (two cases).

A total of 80 of the 115 AAAs assessed (70 per cent) have undergone surgery. Seven patients died within 30 days of operation, two of whom underwent emergency repair for a leaking AAA. None of these patients had an AAA that was

Table 1 Absolute contraindications to endovascular repair in 63 aortic aneurysms assessed by computed tomography angiography

Absolute contraindication	Number of AAAs with contraindication on CT
Proximal neck < 15 mm	40*
Infrarenal aortic diameter > 28 mm	10
Thoracoabdominal aortic aneurysm (suprarenal aortic diameter > 30 mm)	7
External iliac diameter < 9 mm or > 16 mm	3
Bilateral internal and external iliac aneurysms	1
Prosthetic graft material in both groins	1
Evidence of retroperitoneal leak on CT	1

CT, computed tomography. *In 12 abdominal aortic aneurysms (AAAs) the infrarenal aortic diameter was also > 28 mm

Table 2 Relative contraindications to endovascular repair in 13 patients

Relative contraindication	Number
Iliac tortuosity	7
Proximal neck angulation > 60°	4
Bilateral common and internal iliac aneurysms	1
Iliac artery occlusive disease	1

Table 3 Outcome in 35 patients assessed for abdominal aortic aneurysm repair who have not yet undergone surgery

Morphology	Reason for witholding surgery	Number
Suitable for EVAR	Awaiting EVAR	1
	Inappropriate for surgery	
	AP diameter < 5·5 cm	5
	Excessive operative risk	2
	Refused surgery	2
	Awaiting open operation	1
Not suitable for EVAR	Unfit for open AAA repair	11
	Thoracoabdominal AAA	7
	Under observation	4
	Refused surgery	1
	Fatal stroke before surgery	1

AP, anteroposterior; AAA, abdominal aortic aneurysm; EVAR, endovascular repair

morphologically suitable for endovascular repair, and there were no postoperative deaths in the 31 patients who underwent open repair despite being morphologically appropriate for EVAR. The mortality rate was 9 per cent overall for this series, with a 6 per cent mortality rate in patients undergoing elective surgery. The outcome in the 35 patients who have not undergone AAA repair is summarized in *Table 3*.

Discussion

Previous reports suggesting that up to 60 per cent of AAAs would be suitable for EVAR have all originated from centres with a large tertiary referral practice and a declared interest in the development of EVAR[3,7,8]. More recently, other tertiary referral centres have found that the percentage of aneurysms suitable for EVAR is similar to the lower figure observed in the present series[12,13]. However, these figures were based on the use of bifurcated devices alone and it was suggested that the aortouni-iliac configuration may enable EVAR to be undertaken in more than 50 per cent of AAAs[13]. By looking at all AAA referrals in a geographically isolated population of 400 000 the confounding effects of a tertiary referral practice were excluded and yet no more than 30 per cent of AAAs were entirely suitable for EVAR, despite the versatility offered by deploying aortouni-iliac devices in conjunction with femoro-femoral crossover grafting. The inclusion of patients with suboptimal AAA morphology, but no absolute contraindication to EVAR, only increased the suitability to 41 per cent of referrals, provided that currently accepted indications for elective AAA repair were followed.

A recent American report has suggested that as few as 15 per cent of AAAs are suitable for EVAR[14] using the EVT (Endovascular Technologies, Menlo Park, California, USA) device in tube or aortobi-iliac configuration alone. This device fits a maximum aortic diameter of 26 mm. The present study assumed both the availability of aortouni-iliac devices, and devices with a maximum aortic diameter of 31 mm that are available from some manufacturers. The need to oversize endovascular devices to allow for inaccuracy in measuring techniques and to prevent proximal endoleak if the infrarenal neck dilates[15] effectively precludes the use of EVAR where the proximal infrarenal aortic diameter exceeds 28 mm. Custom-made devices to enable the use of EVAR where aortic diameter exceeds 28 mm are available but, with reports of significant annual growth[16], many vascular surgeons would question the wisdom of deploying devices in an infrarenal aorta of this size. There are a few reports of continued infrarenal aortic neck dilatation following both open[17] and endovascular AAA repair[15,18] that raise concerns about long-term fixation of endovascular devices, particularly when deployed in larger aortas. It has been suggested, however, that postoperative dilatation is a consequence of deliberate oversizing of endovascular devices and is not progressive[19], making it less likely to lead to device migration in future years.

In this series it may have been possible to increase the number of aneurysms suitable for EVAR to 40 per cent by attempting to deploy devices in the presence of a number of recognized relative contraindications[2]. The commonest was iliac vessel tortuosity but this is likely to become a less important contraindication as centres increase their experience of EVAR. These suboptimal situations, such as excessively tortuous or aneurysmal iliac vessels and aortic neck angulation, may lead to difficulties in device deployment that could require conversion to open repair. The long-term outcome of deploying devices in these suboptimal situations is unknown; the early endovascular implantations all took place in ideal AAA morphology.

The commonest contraindication to EVAR in the present series was inadequate length of normal infrarenal aorta to enable a good proximal seal. Policy in this unit is not to deploy devices where the infrarenal neck is less than 15 mm in length, although there are devices that have a suprarenal component for proximal anchoring[20]. There is still thought to be a requirement for a reasonable length of normal infrarenal aorta to ensure a good seal[12].

More than half of the AAAs suitable for EVAR in this series could have been treated with the deployment of a bifurcated device. A number, however, would have required distal attachment to one or both external iliac arteries with preoperative or peroperative coiling of the internal iliac arteries to minimize the risk of endoleak and continued aneurysm growth. In addition to the requirement for modular devices with appropriate extension limbs and a risk of significant pelvic ischaemia, this approach significantly increases the operative time, radiation exposure and cost of the technique. The alternative approach of deploying an aortouni-iliac device overcomes some of these problems, and is particularly attractive when iliac angulation is severe, but has the drawback that stenosis or occlusion of the device limb threatens both legs.

In the absence of long-term follow-up data, policy in this unit is to reserve EVAR for patients at increased risk from open repair. In this series, therefore, devices were deployed in fewer than 25 per cent of patients suitable for EVAR; the remainder underwent elective open repair.

The mortality rates observed in this series are comparable with those reported elsewhere[21,22]. Despite restricting the use of EVAR to 'high-risk' patients, no death has been observed following open repair of an AAA that was morphologically suitable for EVAR. While this may be due to careful patient selection for AAA surgery, it is more likely to reflect the fact that the aneurysm morphology favouring EVAR also favours a straightforward open operation. Conversely, factors that preclude the use of EVAR tend to increase technical problems during open repair.

The availability of EVAR has encouraged the assessment of patients for AAA repair who would not be considered appropriate candidates for open aortic surgery. This raises the concern that EVAR might result in surgeons offering intervention to a group of patients with an AAA who would previously have been treated conservatively. The desire to apply this new technology as widely as possible may result in the deployment of endovascular devices in suboptimal morphology which could have adverse sequelae in the longer term. Otherwise, the present study indicates that only about one-third of unselected AAAs are likely to be appropriate for EVAR. The majority of these patients can be treated with a bifurcated device. Most patients suitable for EVAR can safely undergo open aortic surgery.

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Original article

Training in infrainguinal bypass surgery for severe leg ischaemia

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Background: Recent changes in surgical training in the UK mean that operative experience must be gained more efficiently. However, it is important to demonstrate that improved training opportunities are not associated with inferior patient outcomes. The aim was to examine changes in training in infrainguinal bypass surgery and to compare the outcomes of operations performed by consultants and trainees.

Methods: A prospectively gathered, computerized database of 1077 consecutive infrainguinal bypasses performed on 1003 patients for chronic severe leg ischaemia between 1 January 1983 and 31 December 1998 was analysed.

Results: Consultants performed 733 (68 per cent) infrainguinal bypasses to the following distal sites: 347 (47 per cent) above-knee popliteal artery, 257 (35 per cent) below-knee popliteal artery, 121 (17 per cent) to a crural artery and eight (1 per cent) other. Trainees performed 344 operations: 170 (49 per cent) were to the above-knee popliteal artery, 122 (35 per cent) to the below-knee popliteal artery, 48 (14 per cent) crural and four (1 per cent) other. The operative mortality rate was 27 (4 per cent) of 733 for consultants and 11 (3 per cent) of 344 for trainees ($P > 0{\cdot}05$, χ^2 test). There were no significant differences in patency or limb salvage at 36 months after operation between consultants and trainees, regardless of the site of distal anastomosis and the type of conduit used.

Conclusion: With appropriate case selection and supervision, training in infrainguinal bypass grafting does not compromise early or long-term patient outcomes.

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Introduction

Recent changes in surgical training in the UK mean that operative experience must be acquired more quickly and efficiently than before. However, it is important to demonstrate that improved training opportunities are not associated with deteriorating patient outcomes. It has been shown previously that, with adequate supervision, surgeons can be trained in carotid endarterectomy[1] and abdominal aortic aneurysm repair[2] without any increase in 30-day stroke and/or mortality rates. To date, no group has examined the effect of training on the outcome of infrainguinal bypass grafting, where not only operative morbidity and mortality but also long-term patency and limb salvage are important outcome indicators. The aim of this study was to examine changes in training in this unit and to determine what effect, if any, these have had on outcome.

Patients and methods

Analysis of a prospectively gathered, computerized database provided operative and in-hospital outcome data relating to 1077 consecutive infrainguinal bypasses performed on 1003 patients for severe leg ischaemia between 1 January 1983 and 31 December 1998.

Most 'trainee' data relate to the senior registrar grade; the first Calman 'specialist registrar' came to the unit in 1996. A supervised operation refers to when a consultant was in theatre scrubbed and assisting the trainee for all or part of the procedure. An unsupervised operation means that, although always available for advice, the consultant was not scrubbed for any part of the operation. Operative mortality was defined as death within 30 days and/or the same hospital admission. All patients undergoing surgery were followed in a dedicated clinic where outcomes were assessed clinically, judged on symptoms and/or requirement for major limb amputation, as well as haemodynamically by means of ankle : brachial pressure index. Patency of vein bypasses was assessed at 6 weeks and 3, 6, 9, 12 and 24 months after operation by duplex ultrasonography as part of a graft surveillance programme. When a graft was believed to have occluded on clinical and/or haemodynamic grounds, this was confirmed or refuted by duplex ultrasonography and/or angiography.

Primary patency and limb salvage were analysed by life-table methods, and differences between groups were tested for significance with the log rank test. $P < 0.05$ was considered statistically significant.

Results

During the 16 years studied, 1077 infrainguinal bypasses were performed on 1003 patients: 733 (673 patients) by consultants and 344 (330 patients) by trainees. Trainees were supervised in 45 (13 per cent) and unsupervised in 299 (87 per cent) operations. During the first 8 years of the study, trainees performed 87 (17 per cent) of 522 operations, compared with 257 (46 per cent) of 555 in the second 8 years ($P < 0.0001$, χ^2 test).

There was no significant difference between operations done by consultant or trainee with regard to the site of the distal anastomosis, the conduit used (*Table 1*) or perioperative mortality rate (27 of 733 (4 per cent) consultants *versus* 11 of 344 (3 per cent) trainees). Median (range) follow-up for operations done by consultant was 24 (1–181) months, compared with 21 (1–115) months for those done by trainees (*P* not significant). Primary graft patency and limb salvage rates after 36 months were similar in both groups (*Figs 1* and *2*).

Table 1 Characteristics of infrainguinal bypass operations done by consultants or trainees

Distal anastomosis	*n*	Synthetic bypass	Synthetic and vein cuff	Vein bypass
Consultants				
Above knee	347 (47)	147	18	182
Below knee	257 (35)	66	44	147
Crural	121 (17)	16	21	84
Other	8 (1)	5	0	3
Total	733 (100)	234	83	416
Trainees				
Above knee	170 (49)	77	9	84
Below knee	122 (35)	22	32	68
Crural	48 (14)	5	13	30
Other	4 (1)	1	1	2
Total	344 (100)	105	55	184

Values in parentheses are percentages

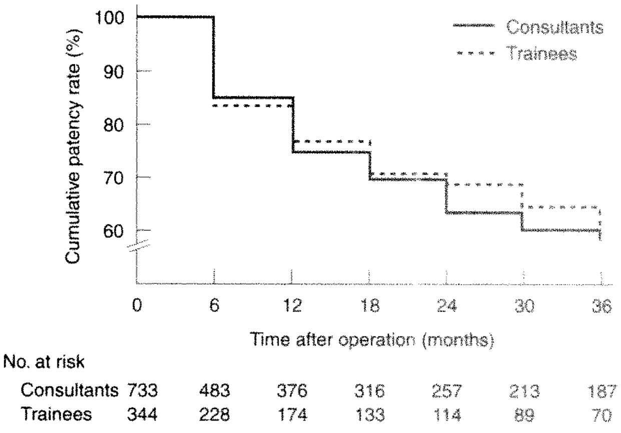

Fig. 1 Cumulative primary patency of infrainguinal grafts done by consultants and trainees. The standard error was less than 5 per cent throughout the follow-up

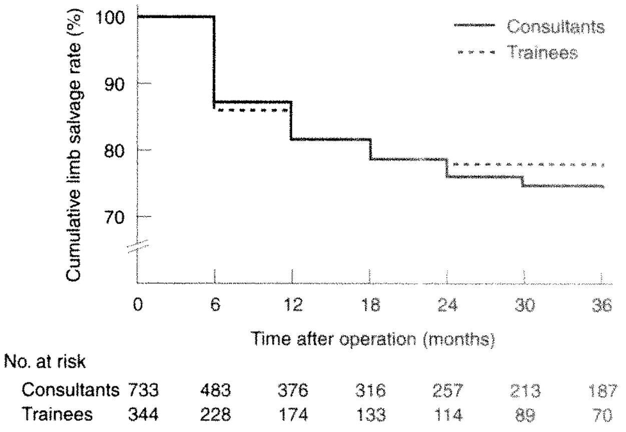

Fig. 2 Cumulative limb salvage after infrainguinal bypass grafts done by consultants and trainees. The standard error was less than 6 per cent throughout the follow-up

Discussion

In the UK, the reduction in working hours together with the introduction of the specialist registrar grade has reduced the duration of surgical training[3] from 80 000 to 25 000 h. Experience, particularly relating to operative skills, has to be acquired much more quickly and efficiently if standards are to be maintained[4–7]. Improved training opportunities must not be achieved at the expense of inferior patient outcomes.

With regard to vascular surgery, aortic reconstruction, carotid endarterectomy and infrainguinal bypass surgery have been proposed as indicator or 'bench-mark' operations[3]. Previously published data from this unit have demonstrated that, with adequate supervision, surgeons can be trained in carotid endarterectomy[1] and abdominal aortic aneurysm repair[2] without any increase in 30-day stroke and mortality rates.

The principal finding of this study is that, despite a highly significant increase in the number of procedures performed by trainees, there has been no increase in operative mortality rate and no reduction in patency and limb salvage rates at 36 months. Furthermore, this has been achieved without 'protecting' trainees from the more challenging crural bypass procedures. However, it must be remembered that these data relate largely to a time when experienced senior registrars, used to working independently, were overseen by senior consultants. Whether the same training opportunities and patient outcomes can be achieved under the Calman system remains to be seen. With appropriate case selection and supervision, training in infrainguinal bypass grafting does not compromise patient outcome.

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Original article

Experimental sodium phosphate and polyethylene glycol induce colonic tissue damage and oxidative stress

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Background: Bowel washout solutions may damage colonic mucosa and cause shallow ulceration, which may result in diagnostic errors and complications. The effects of polyethylene glycol (PEG) and sodium phosphate on rat colon were investigated histologically and by measurement of indicators of oxidative stress.

Methods: Thirty Wistar albino rats were divided into three groups and received PEG, sodium phosphate or tap water alone (control). After 8 h, histological changes in colonic mucosa were evaluated. The tissue concentration of malonyldialdehyde (MDA), superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) in colon homogenates was also measured.

Results: Animals in the sodium phosphate and PEG groups had significantly more colonic mucosal damage than controls, the damage induced by sodium phosphate being worse than that caused by PEG. MDA levels were significantly higher in the sodium phosphate and PEG groups than in controls and were higher in the sodium phosphate group than in the PEG group, whereas differences in SOD and GSH-Px activities were significant only between control and both sodium phosphate and PEG groups.

Conclusion: Sodium phosphate and PEG cause histological damage and trigger oxidative stress on colonic mucosa. The tissue damage and oxidative stress induced by sodium phosphate is more profound than that produced by PEG.

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Introduction

Adequate bowel preparation has become the standard of care in the preoperative preparation of patients for colonic surgery and colonoscopy. Three-day cathartic regimens were used for many years in the past, but currently 1 day of treatment with oral solutions is the most common method of mechanical bowel cleansing. Polyethylene glycol (PEG) and sodium phosphate are the most commonly used agents for this purpose. Although mechanical colon cleansing effectively reduces the incidence of anastomotic breakdown and wound infections after colonic surgery and provides excellent visualization for colonoscopy[1,2], occasionally complications occur such as electrolyte disturbances and patient discomfort[3,4]. Recently, mucosal damage as a result of the use of colon cleansing agents has been observed, and sodium phosphate solution in particular has been reported to cause aphthoid-like lesions on the colonic mucosa, which may be mistaken for a disease process[5]. Although lesions induced by colon cleansing agents have been reported by several investigators[5–7], the mechanism for this mucosal damage is not clear.

Oxidative stress is known to play a role in the pathophysiology of many conditions. In a living organism, free radicals are inevitably generated and perfectly balanced by the antioxidant system. Whenever the balance shifts in favour of oxidants, oxidative stress and therefore tissue damage occurs[8]. It was hypothesized that oxidative stress may have a role in the mucosal changes associated with colon cleansing.

In this study the effects of the colon cleansing solutions sodium phosphate and PEG on rat colonic mucosa were examined histologically, and their effects on endogenous oxidant–antioxidant activity were studied.

Materials and methods

Animals

Local ethics committee approval was obtained before starting the experiments. Thirty male Wistar albino rats weighing 200–260 g were divided randomly into three groups. All rats were fed with standard pelleted food, had free access to water and were housed in cages at a controlled temperature and circadian light–dark cycle. Sodium phosphate (1·5 ml/kg) was given to rats in the sodium phosphate group and PEG (66 ml/kg) was administered to animals in the PEG group by gavage. Animals in the control group were given water to drink. After 8 h all rats were killed. The abdomen was opened and tissue samples of colon above the pelvic peritoneum were taken.

Histological evaluation

For histological examination samples were fixed in formalin and embedded in paraffin blocks. Sections were stained with haematoxylin and eosin, and evaluated by light microscopy. Microscopic studies were reviewed by one blinded observer (an experienced pathologist). Sections were examined specifically for loss of superficial mucosa, epithelial denudation, oedema in the lamina propria, submucosal congestion, neutrophilic infiltration, loss of goblet cells and preulcer lesions, which have been described previously by Vanner *et al.*[9] (*Table 1*).

Biochemical assay

Determination of oxidant activity

Colonic tissues were homogenized in phosphate buffer 0·1 mol/l (pH 7·4). The malonyldialdehyde (MDA) concentration in colon homogenate was determined with the thiobarbituric acid (TBA) method[10]. Briefly, 3 ml 1 per cent phosphoric acid and 1 ml 0·67 per cent TBA solution were added to a 0·5-ml tissue sample. The mixture was heated in boiling water for 1 h and, after cooling, 4 ml 1-butanol were added and mixed vigorously. After centrifugation the butanol phase was removed and absorbance at 532 nm was used to calculate the MDA concentration. The MDA standard was prepared from 1,1,3,3-tetramethoxypropane. The MDA concentration in colon homogenate was expressed in relation to protein concentration, which was measured by the method of Lowry *et al.*[11]. The results were expressed as nanomoles per milligram of protein.

Determination of antioxidant activity

The homogenate was centrifuged at 15 000*g* for 60 min. Clear upper supernatant fluid was obtained for the enzyme assays. Glutathione peroxidase (GSH-Px) activity in the supernatant of colon homogenate was determined by using cumene hydroperoxide as substrate according to the method of Paglia and Valentine[12]. The method is based on the reduced nicotinamide–adenine dinucleotide phosphate (NADPH)-coupled reaction by which oxidized glutathione produced by the activity of GSH-Px is converted to reduced glutathione by exogenous glutathione reductase and NADPH. The rate of NADPH oxidation was measured at 340 nm. GSH-Px activity was expressed as micromoles of NADPH oxidized to NADP$^+$ per minute per milligram of protein using an extinction coefficient (6·22 mmol^{-1} cm^{-1}) for NADPH. GSH-Px activity was expressed as units per milligram of protein.

Superoxide dismutase (SOD) activity of the supernatant of colon homogenate was determined with a commercially available kit (Randox Laboratories, Antrim, Ireland). SOD estimation was based on the generation of superoxide radicals produced by xanthine and xanthine oxidase, which reacts with 2-(4-iodophenyl)-3-(4-nitrophenol)-5-phenyltetrazolium chloride to form a red formazan dye. SOD activity was then measured by the degree of inhibition of this reaction, and is expressed as units per milligram of protein.

Statistical analysis

Data are presented as median (95 per cent confidence interval). Comparisons of the median values for MDA, SOD and GSH-Px between groups were made with the Kruskal–Wallis one-way analysis of variance. Comparison

Table 1 Frequency of histopathological variables in the three groups

Histological feature	Control (*n* = 10)	Sodium phosphate (*n* = 10)	PEG (*n* = 10)	χ^2	d.f.	*P*
Loss of superficial mucus	3	10*	10*	18·26	2	0·0001
Epithelial denudation	1	10*	8*	19·23	2	0·0001
Oedema in lamina propria	0	9*	5*†	16·34	2	0·0001
Submucosal congestion	0	5*	2	7·08	2	0·03
Neutrophilic infiltration	0	3	1	4·04	2	0·13
Loss of goblet cells	0	2	0	4·29	2	0·12
Preulcer lesions	0	1	0	2·07	2	0·36

PEG, polyethylene glycol. *$P < 0·05$ *versus* control group (χ^2 test), †$P < 0·05$ *versus* sodium phosphate group (χ^2 test) of the frequency of histopathological variables among groups was performed with the χ^2 test with Tukey's b correction. Differences were considered statistically significant when $P<0{\cdot}05$.

Results

Histopathological findings

All samples were examined by light microscopy. In the sodium phosphate group, loss of superficial mucus and epithelial denudation were seen in all ten samples, and oedema in the lamina propria and submucosal congestion were seen in nine and five samples respectively. The same changes were observed in the PEG group in ten, eight, five and two of the ten samples respectively. However, in the control group loss of superficial mucus was seen in only three samples and epithelial denudation in one. Other histological changes are summarized in *Table 1*. *Figs 1* and *2* show representative histological sections from the sodium phosphate and PEG groups.

Oxidative stress

Levels of MDA, an indicator of increased oxidant activity, were significantly higher in the sodium phosphate and PEG groups than in controls. The difference between the sodium phosphate and PEG groups was also significant (*Table 2; Fig. 3*).

Levels of both SOD and GSH-Px, indicators of the antioxidant system, were significantly higher in the sodium phosphate and PEG groups than in controls. However, differences in SOD and GSH-Px levels between the sodium phosphate and PEG groups were not significant (*Table 2; Fig. 3*).

Discussion

Although mechanical colon cleansing before colonic surgery, colonoscopy and barium enema has become standard, this procedure is not free of complications. Many studies have reported systemic side-effects such as electrolyte disturbance and gastrointestinal discomfort[13,14].

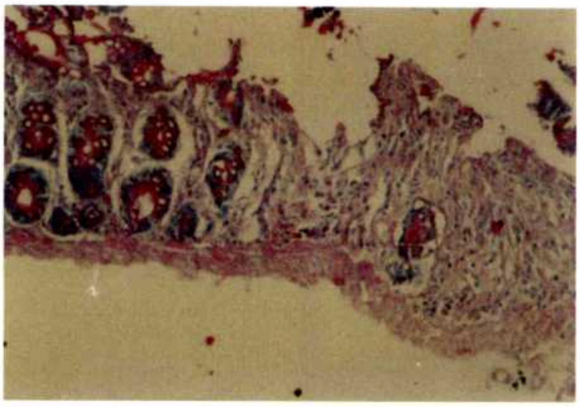


Fig. 1 Histological section of a sodium phosphate-induced lesion. Superficial epithelial denudation and goblet cell loss are seen. This appearance is termed a preulcer lesion in colonic submucosa. (Haematoxylin and eosin stain; original magnification × 100)

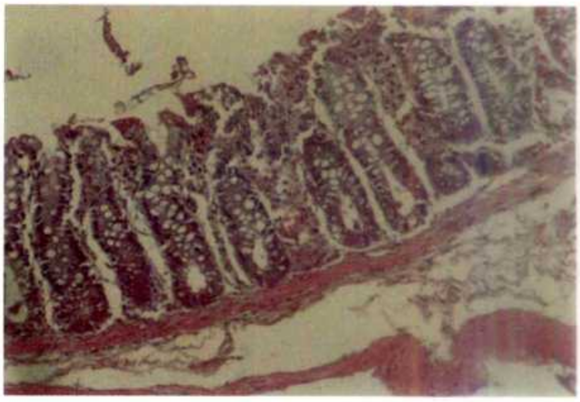
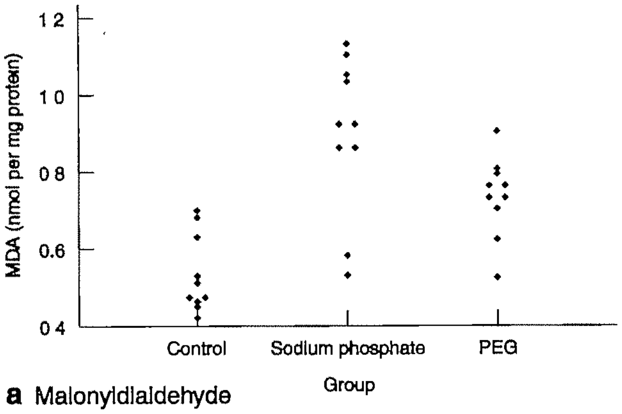


Fig. 2 Histological section of a polyethylene glycol-induced lesion. Superficial mucus loss and denudation of superficial epithelium are noted. However, goblet cells appear to be better preserved compared with the sodium phosphate group. (Haematoxylin and eosin stain; original magnification × 100)

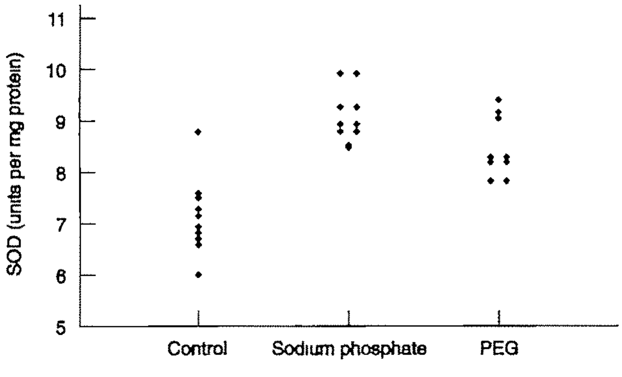
Table 2 Malonyldialdehyde, superoxide dismutase and glutathione peroxidase levels in the three groups

	Control ($n=10$)	Sodium phosphate ($n=10$)	PEG ($n=10$)	P
MDA (nmol/mg protein)	0·53 (0·46–0·60)	0·89 (0·74–1·05)*	0·73 (0·66–0·80)*†	0·0003
SOD (units/mg protein)	7·1 (6·6–7·7)	9·1 (8·7–9·5)*	8·6 (8·1–9·1)*	0·0002
GSH-Px (units/mg protein)	126 (108–145)	165 (156–175)*	150 (137–165)*	0·003

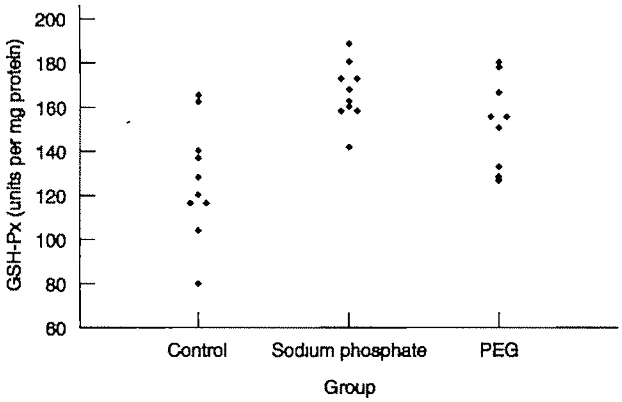
Data are median (95 per cent confidence interval). MDA, malonyldialdehyde; SOD, superoxide dismutase; GSH-Px, glutathione peroxidase. $^*P<0{\cdot}05$ *versus* control group, $^\dagger P<0{\cdot}05$ *versus* sodium phosphate group (Kruskal–Wallis one-way analysis of variance)



a Malonyldialdehyde



b Superoxide dismutase



c Glutathione peroxidase

Fig. 3 Scattergrams showing the distribution of a malonyldialdehyde (MDA), b superoxide dismutase (SOD) and c glutathione peroxidase (GSH-Px) activity in control, sodium phosphate and polyethylene glycol (PEG) groups

Recently, the effects of cleansing solutions on colonic mucosa have been reported. Hixson[15] has shown that sodium phosphate produced aphthous lesions in colonic and rectal mucosa. Similar mucosal abnormalities have been reported in as many as 24 per cent of patients with sodium phosphate and 2·3 per cent with PEG[5]. These abnormalities may cause diagnostic errors and complications.

Although studies have revealed macroscopic colonic mucosal abnormalities, the histological features are unknown. Nevertheless, it is obvious that damage that can be detected microscopically would be of greater value than macroscopic findings[16]. In this histological study, the most prominent characteristics in all samples from the sodium phosphate and PEG groups were loss of superficial mucus and mucosal denudation. Other changes indicating more severe tissue damage, such as tissue oedema, loss of goblet cells and preulcer lesions, were also seen in some samples. However, there were no appreciable macroscopic differences between the groups (data not shown).

The molecular mechanism of lavage-induced mucosal damage has not been well investigated. The present results show that both oxidants and antioxidants are stimulated by colon cleansing with sodium phosphate and PEG, suggesting that oxidative stress is involved in colonic mucosal damage induced by these compounds. Oxidative stress is a potentially harmful event that causes tissue damage unless the oxidants are kept in balance by the antioxidant system[17]. Free oxygen radicals are highly reactive and unstable chemical compounds with one or more unpaired electrons, allowing them to react with vital components of cells, particularly membrane lipids, a process called lipid peroxidation[18]. Membrane lipid peroxidation yields MDA, which is accepted as an early indicator of oxidative damage and cell death[19]. The finding of higher MDA levels in colonic tissue from the sodium phosphate and PEG groups than in that from controls suggests the possible role of oxidative stress in this process.

The observation of increased SOD and GSH-Px activity in experimental groups indicates that the antioxidant system is also activated by colon cleansing by sodium phosphate and PEG solutions. This result points indirectly to the involvement of oxidative stress in this process rather than indicating that these compounds trigger the antioxidant system directly. Previous studies have shown that antioxidants are activated constantly whenever oxidative stress occurs unless the antioxidative capacity is attenuated by chronic disorders[20]. Therefore, some degree of antioxidant activation is to be expected in acute conditions such as colonic mucosal epithelial distress resulting from cleansing solutions.

It is difficult to explain how colon cleansing disturbs the oxidant–antioxidant balance. Neutrophilic infiltration was seen in tissue sections from some samples from the sodium phosphate and PEG groups. Neutrophils infiltrating the inflamed colonic mucosa have been reported to produce superoxide, hydroxyl radicals and hydrogen peroxide[21,22]. Thus, one possible explanation may be that free radicals generated by abundant neutrophils situated in submucosa violate the oxidant–antioxidant balance. However, neutrophilic infiltration was not observed in all samples from the sodium phosphate and PEG groups, suggesting the need for further research.

Histological damage and MDA production were greater in the sodium phosphate group than in the PEG group. Although, clinically, less gastrointestinal discomfort has been reported with sodium phosphate than with PEG[23], from the histological and biochemical viewpoints PEG appears to be less harmful to the colonic mucosa than sodium phosphate.

In conclusion, these results clearly show that two commonly used colon cleansing solutions, sodium phosphate and PEG, produce histological damage and trigger oxidative stress and increased free radical production in colonic mucosa. Tissue damage and oxidative stress induced by sodium phosphate was greater than that produced by PEG. It is suggested that these agents should be used carefully and that physicians must be aware of such consequences. Efforts to reduce the side-effects of colon cleansing methods need to be encouraged.

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Original article

Insulin-like growth factor I and truncated keratinocyte growth factor accelerate healing of left-sided colonic anastomoses

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Background: Human full-length keratinocyte growth factor (KGF) promotes healing of colon anastomoses in rats through mechanisms other than enhancement of collagen synthesis. Since insulin-like growth factor (IGF) I increases matrix synthesis, the aim of this study was to evaluate the effect of systemic truncated KGF (tKGF), IGF-I and combined tKGF–IGF-I administration on the healing of colonic anastomoses in rats.

Methods: Rats underwent laparotomy, division of the left colon, and sigmoidosigmoidostomy. tKGF (1 mg/kg), IGF-I (1 mg/kg), tKGF–IGF-I (both 1 mg/kg) or vehicle was administered intraperitoneally in four groups ($n = 18$ per group) 12 h before surgical intervention, and then once daily until killing (six animals per group; 2, 4 and 6 days after surgery). Bursting pressure measurements, histological evaluation, morphometric analysis, mucin and collagen staining, and 5-bromo-2′-deoxyuridine (BrdU) immunohistochemistry of the anastomotic site were undertaken.

Results: Administration of tKGF, IGF-I and the combination of both growth factors significantly increased anastomotic bursting pressure at postoperative day 2 (63, 71 and 113 per cent respectively), day 4 (68, 83 and 80 per cent) and day 6 (48, 43 and 43 per cent) compared with the control group. No intergroup differences were found. Histological examination, mucin and BrdU staining, and measurement of colonic crypt depth indicated less inflammation, increased acidic mucin content, a higher crypt cell proliferation rate and thickened mucosal layer in the growth factor-treated animals than in controls. Enhanced collagen staining was observed only in IGF-treated animals.

Conclusion: tKGF and IGF-I markedly accelerate the healing of colonic anastomoses in rats. However, combined administration of the two growth factors does not show additional benefit. Both growth factors may be acting to accelerate host reparative processes as well as to enhance protection of the anastomotic wound bed.

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Introduction

Anastomotic dehiscence following colorectal surgery is a significant cause of morbidity and mortality[1,2]. The rate of clinically apparent anastomotic leakage ranges from 3·4 per cent[1] to 8 per cent[3,4]. At least one-third of deaths following colorectal surgery are attributed to anastomotic leakage[1,3–5]. The effects of growth hormone and growth factors on the healing of bowel anastomosis are currently being evaluated in an attempt to identify agents that may promote the anastomotic healing process.

Keratinocyte growth factor (KGF), synthesized and secreted by stromal fibroblasts, is expressed predominantly in the dermis[6]. However, expression of KGF receptor and KGF messenger RNA (mRNA) has been detected throughout the entire gastrointestinal tract[7]. Recent studies support a role for KGF in tissue repair[8,9]. In inflammatory bowel disease, increased expression of KGF has been described in the mucosa, suggesting a potential role for KGF in mediating mucosal repair mechanisms[10,11]. It also has been shown that systemic administration of KGF ameliorates colonic mucosal injury in different models of colitis in rats and mice[12,13]. In addition, systemic administration of KGF promotes anastomotic healing in the left colon in rats[14] and increases anastomotic bursting pressure compared with values in control animals. However, the collagen content of anastomoses was not significantly different in animals given KGF or vehicle. These results suggest that KGF favourably affects the rate of early anastomotic healing through mechanisms other than enhancement of collagen synthesis.

Insulin-like growth factor (IGF) I is a peptide hormone structurally related to insulin which has pleotrophic effects on cell growth and metabolism. Exogenous administration of the peptide has been shown to induce impressive intestinal growth in dexamethasone-treated rats[15] and to enhance mucosal adaptation after jejunoileal resection[16]. Furthermore, intra-ileal infusion of IGF-I has been shown to have trophic effects on the intestine[17]. IGF-I receptors have been characterized throughout the gastrointestinal tract in several species[18] and there is also a marked upregulation of IGF-I binding sites in experimental colitis in rats[19]. IGF-I also partially prevents experimental colitis in rats[20]. IGF-I has been demonstrated to reverse diabetes-induced impairment of wound healing in rats[21].

So far, very little is known about the role of IGF-I in anastomotic healing. Petersen *et al.*[22] were able to demonstrate that systemic IGF-I administration markedly increased anastomotic collagen content early after surgery. IGF-I did not seem to increase the mechanical stability of colonic anastomosis measured by breaking strength, compared with untreated animals[22]. However, the bursting pressure of anastomotic sites is not directly related to the anastomotic collagen content during the early healing phase[23]. It may therefore be that IGF-I affects the mechanical stability of the anastomosis later in the postoperative period and not as early as the first postoperative day.

Based on these findings, the combined administration of KGF, with effects mainly on mucosal epithelial cells, and of IGF-I, with possible effects on extracellular matrix production, could be an ideal combination to promote healing of colonic anastomoses. Because full-length KGF was not available, a truncated form of the growth factor (tKGF; Chiron, Emeryville, California, USA) was used in this study.

Materials and methods

Ethical considerations

The Animal Care and Use Review Committee of the University of Berne approved the study in accordance with standards set out in the Animal Welfare Act and other Federal, State and Local Statutes and Regulations related to animals.

Truncated keratinocyte growth factor and insulin-like growth factor I

Purified tKGF and human recombinant IGF-I were manufactured and provided by Chiron.

Evaluation of the effect of truncated keratinocyte growth factor on colonic mucosa

Pilot studies were performed to evaluate the effectiveness of tKGF on colonic mucosa. Eighteen male Sprague–Dawley rats, weighing 280–300 g, were utilized in these studies. Animals were divided into six groups of three animals. A different dose of tKGF (1·2, 0·6, 0·3, 0·15 mg/kg) or vehicle was administered intraperitoneally once daily in each group. Furthermore, normal saline (0·9 g/per cent) was administered in additional control animals accordingly. On the seventh day animals were killed and histological, morphometric and immunohistochemical evaluations were performed as described below.

Animals and administration of growth factors

Eighty male Sprague–Dawley rats, weighing 280–300 g, were used in these studies. They were housed individually and fed a standard laboratory diet and water *ad libitum*. Animals were fasted for 12 h before the surgical intervention. They were divided into four groups ($n = 18$ in growth factor groups; $n = 24$ in the vehicle group). tKGF (1 mg per kg body-weight – dose derived from the results of the pilot study), IGF-I (1 mg per kg body-weight – twice the dose used by Petersen *et al.*[24]), the combination of both growth factors, or vehicle was administered intraperitoneally once daily until killing (2, 4, 6 and 8 days after operation; six animals from each group at each time point). Treatment was initiated 12 h before operation.

Operative technique

Operative technique was as described previously[14]. After dissection of the bowel, anastomosis was performed using microsurgical techniques in an inverted and interrupted fashion with 6/0 polydioxanone sutures (PDS II; Ethicon, Somerville, New Jersey, USA).

Bursting pressure technique

Bursting pressure was evaluated with investigators blinded to the test animals, as described previously[14]. After dissection of the anastomotic site and submersion in a saline bath, bursting pressure was measured by means of a sphygmomanometer with an inline pressure transducer by increasing the intraluminal pressure in increments of 10 mmHg over 10 s at intervals of 10 s. Bursting pressure was determined by noting leakage of air or gross rupture of the anastomosis or another part of the bowel segment.

Histological assessment and morphometric analysis

The segment of bowel was opened longitudinally, fixed in 10 per cent zinc–formalin and embedded in paraffin. Transverse sections of the embedded tissue were stained with haematoxylin and eosin, and histological assessment was carried out. A computed measurement of the thickness of the colonic crypts (colonic crypt depth) was performed. Ten random measurements were performed at least 2 mm from the anastomotic site where glands were perpendicular to the underlying muscularis. Inflammatory activity at the anastomotic site was scored (from 1+ to 3+), blinded to the treatment group, based on the extent of inflammatory tissue and number of inflammatory cells. All results are expressed as the mean per group.

Assessment of proliferating cells using antibromodeoxyuridine

All rats received an intraperitoneal injection of 5-bromo-2′-deoxyuridine (BrdU) 50 mg/kg (Sigma-Aldridge Chemie, Steinheim, Germany) 120 min before killing. Paraffin-embedded sections at standard thickness were deparaffinized and BrdU immunohistochemistry was performed as described previously[14,25]. The total number of proliferating cells per crypt was counted at a magnification of 1 : 100 in ten longitudinal cuts of colonic crypts by two independent investigators blinded to treatment groups. Results were expressed as the total number of BrdU-positive cells per crypt.

Mucin staining

Embedded tissue was cut in 7-μm slices and the slides were subsequently deparaffinized, hydrated to water, and sulphated mucins were stained with Alcian blue at pH 1·0, whereas acidic mucins were stained with Alcian blue at pH 2·5[26]. Neutral mucins were stained with periodic acid-Schiff (PAS)[27].

Elastica–van Gieson stain

Embedded tissue was cut in 7-μm slices and classical Elastica–van Gieson staining was performed[27].

Statistical analysis

Results are expressed as mean(s.e.m.). The significance of differences was assessed with the Mann–Whitney *U* test; $P < 0{\cdot}05$ (two tailed) was considered statistically significant.

Results

Pilot studies

With increasing dose of tKGF, a stepwise increase of the crypt depths (*Fig. 1*), intensity of mucin staining and the number of BrdU-positive cells (*Fig. 2*) was observed. Based on these results, a dose of 1 mg per kg body-weight was used in the main study, which is five times less than the dose used in previous studies with full-length KGF[14].

Two animals (one in the control group, one in the IGF-I group) died during the operative intervention. All other

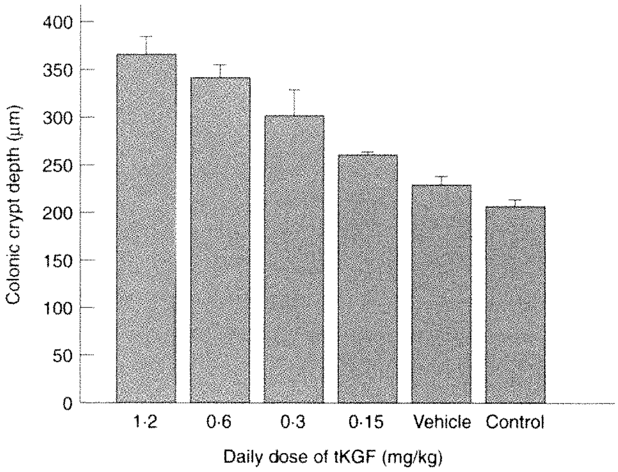

Fig. 1 Colonic crypt depth in unoperated animals treated with truncated keratinocyte growth factor (tKGF) or vehicle and in controls

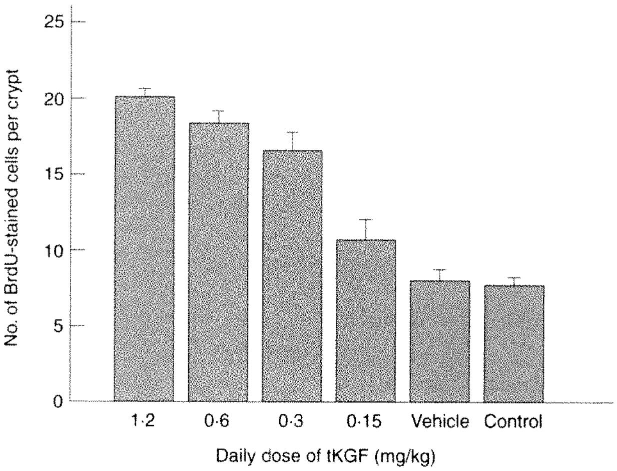

Fig. 2 Number of 5-bromo-2′-deoxyuridine (BrdU)-stained cells per crypt in unoperated animals treated with truncated keratinocyte growth factor (tKGF) or vehicle and in controls

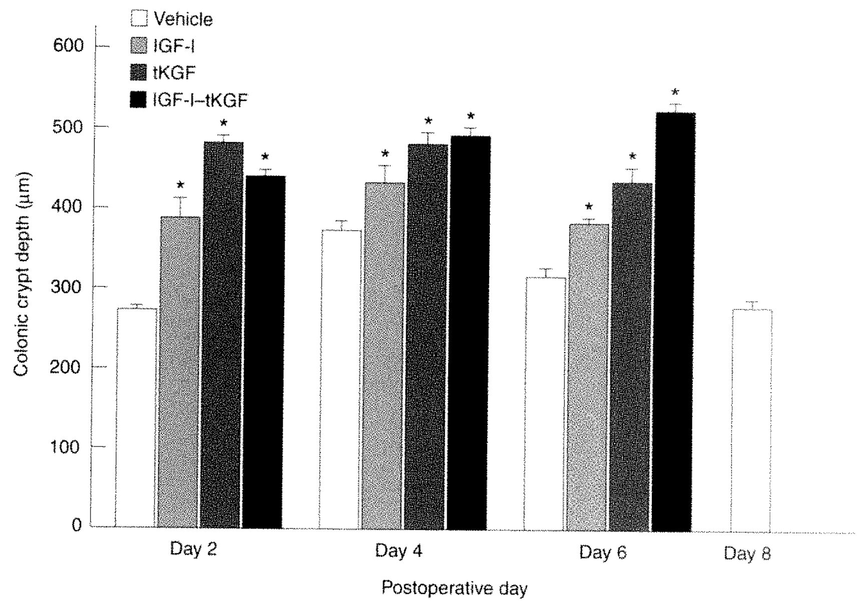

Fig. 3 Colonic crypt depth in rats treated with insulin-like growth factor (IGF) I, truncated keratinocyte growth factor (tKGF), IGF-I–tKGF or vehicle. *$P < 0{\cdot}05$ *versus* vehicle-treated animals (Mann–Whitney *U* test)

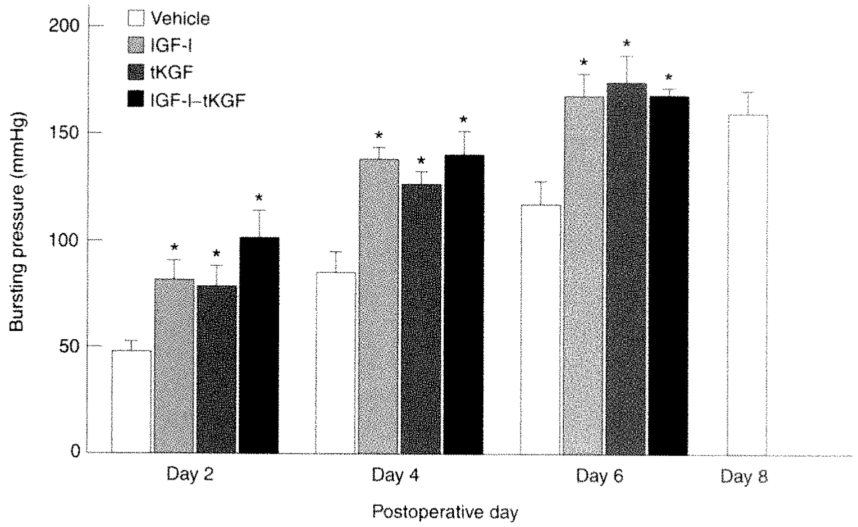

Fig. 4 Bursting pressure of left-sided colonic anastomosis in rats treated with insulin-like growth factor (IGF) I, truncated keratinocyte growth factor (tKGF), IGF-I–tKGF or vehicle. *$P < 0{\cdot}05$ *versus* vehicle-treated animals (Mann–Whitney *U* test)

animals tolerated surgery well and there was no further death during the study.

Macroscopic and microscopic pathological assessment

At necropsy, three contained anastomotic leaks were noted in the vehicle-treated group (one on each of days 2, 4 and 8), although clinically all animals appeared asymptomatic. All leaks were contained with either omentum or small bowel loops and there was no evidence of peritonitis. As anticipated, burst during bursting pressure measurements occurred at the site of the leak.

Macroscopically, the dissected bowel appeared thickened in tKGF- and IGF-I-treated animals as well as in the IGF-I–tKGF group, compared with control (vehicle) animals at each evaluated time point. Computed microscopic measurements of colonic crypt depth revealed a signifi-

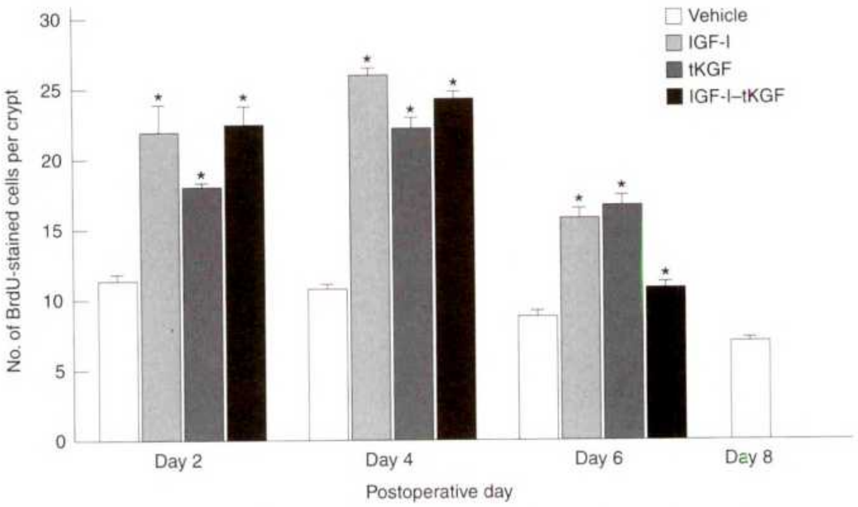

Fig. 5 Number of 5-bromo-2′-deoxyuridine (BrdU)-stained cells per crypt in rats treated with insulin-like growth factor (IGF) I, truncated keratinocyte growth factor (tKGF), IGF-I–tKGF or vehicle. $^*P < 0.05$ *versus* vehicle-treated animals (Mann–Whitney U test)

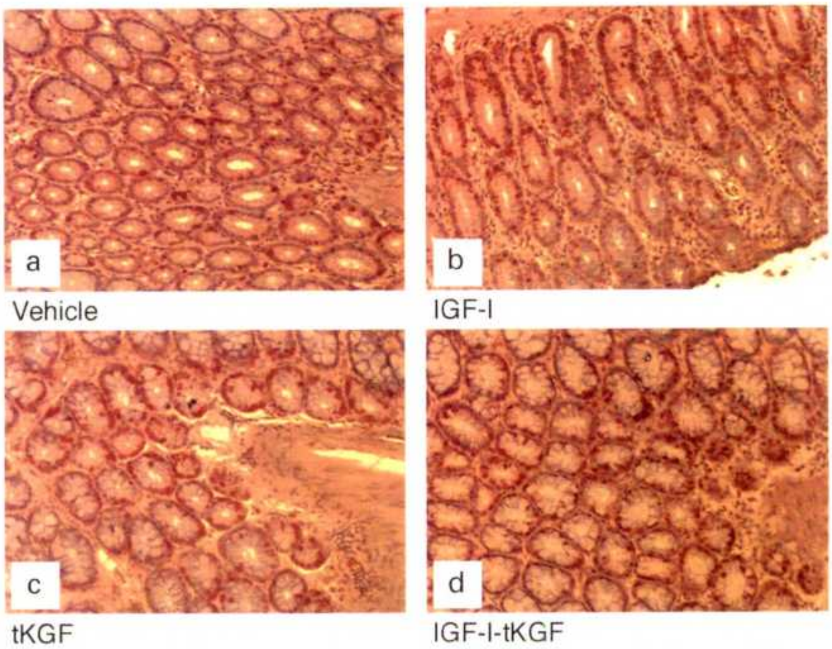

Fig. 6 5-Bromo-2′-deoxyuridine-stained cells in crypts of rats treated with **a** vehicle, **b** insulin-like growth factor (IGF) I, **c** truncated keratinocyte growth factor (tKGF) and **d** IGF-I–tKGF on postoperative day 4 (original magnification × 40)

cant increase in colonic mucosa thickness that correlated with macroscopic findings (*Fig. 3*). Mean(s.e.m.) colonic crypt depth in vehicle animals was 280(9) on day 8. An increase in mucosal thickness of vehicle animals on days 4 and 6 ($P < 0.05$) was found when compared to the value for day 2 or 8. The thickness of the muscularis propria and submucosa layer was no different between the study groups.

The overall evaluated inflammatory scores were 2·56(0·18), 1·22(0·15), 1·56(0·24) and 1·44(0·24) in animals treated with vehicle, IGF-I, tKGF and IGF-I–tKGF respectively ($P < 0.05$ for all test groups compared with

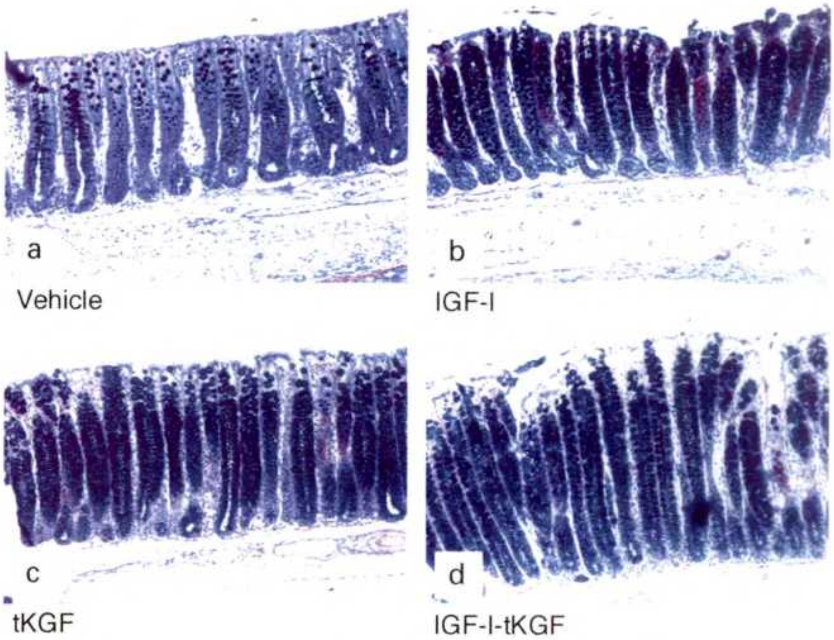

Fig. 7 Mucin staining of colon from rats treated with **a** vehicle, **b** insulin-like growth factor (IGF) I, **c** truncated keratinocyte growth factor (tKGF) and **d** IGF-I–tKGF on postoperative day 4 (original magnification × 40)

control animals at each evaluated time point). Similarly, more micro-abscesses were found in vehicle-treated animals ($n=9$) than in all other groups (IGF-I, four; tKGF, five; IGF-I–tKGF, five) on microscopic evaluation of the anastomotic sites. In both groups, neutrophils were abundant in micro-abscesses, whereas granulation tissue and foreign body reactions were present adjacent to suture material.

Bursting pressure assessment

Intestinal bursting occurred in all animals at or near the anastomotic site at a pressure that was significantly higher in growth factor-treated groups than in controls on postoperative days 2, 4 and 6. The mean(s.e.m.) bursting pressure of vehicle animals killed on day 8 was 161(10) (*Fig. 4*).

Mucosal proliferation

There was a marked increase in the number of BrdU-labelled cells in the growth factor-treated tissue when compared to control tissue on every evaluated day (*Figs 5* and *6*). This increase was localized to the basal and middle thirds of the crypts. There was also a slight increase in proliferating cells in the vehicle group on days 2, 4 and 6 when compared to day 8, when the number of proliferating cells was almost back to the basal level.

Mucin staining

On opening the colonic specimen, mucus content in animals treated with IGF-I, tKGF and IGF-I–tKGF appeared to be increased compared with that in the vehicle group. Staining of acidic/sulphated mucins with Alcian blue and of neutral mucins with PAS revealed a marked increase in the number of goblet cells, as well as enhanced staining for acidic mucin within the goblet cells, in the colon of rats administered IGF-I, tKGF and IGF-I–tKGF in comparison with vehicle-treated animals on postoperative days 2 and 4 and 6 (*Fig. 7*). No change in either the number or the intensity of goblet cell staining for neutral mucin with PAS was identified by growth factor administration on postoperative days 2, 4 and 6. Comparing the groups with growth factor treatment, a stepwise increase was found in rats treated with vehicle, IGF-I, tKGF and IGF-I–tKGF. These findings are reflected in the ratio of acidic : neutral mucins, expressed as the mean per group: 1·4 : 1, 2·2 : 1, 2·6 : 1 and 3·1 : 1 for vehicle, IGF-I, tKGF and IGF-I–tKGF groups respectively.

Elastica–van Gieson stain

On sections stained for connective tissue, the density of collagen fibres at the anastomotic sites in and around the inflammatory granulation tissue on days 2, 4 and 6 was increased in the IGF-I and IGF-I–tKGF groups compared with the vehicle and tKGF groups (data not shown).

Discussion

The results of the present study demonstrate that systemic administration of tKGF and IGF-I significantly accelerates the healing of left-sided colonic anastomoses. tKGF, IGF-I and the combination of both growth factors resulted in higher bursting pressures and hence enhanced stability of colonic anastomosis during the first 6 days after surgery. It appears that vehicle-treated animals achieve the bursting pressures observed in growth factor-treated animals approximately 2 days later. Administration of IGF-I, tKGF or the combination of both growth factors resulted in a marked thickening of the mucosal layer throughout the entire colon. The proliferative effect (or decreased apoptosis) of KGF on colonic mucosa has been demonstrated previously[14]. The results of the BrdU staining in the present study confirm the induction of epithelial proliferation by tKGF and IGF-I with a marked increase in the mitotic activity of mucosal cells, especially at the base of the crypts. However, no beneficial effect was observed for the simultaneous administration of the two growth factors.

A role for KGF as an endogenous mediator of growth and differentiation in the gastrointestinal tract is supported by the isolation of mRNA for KGF and its receptor in both the small and large intestine of rodents[7,28]. Recently, it has been demonstrated that exogenous full-length KGF significantly reduces the severity and extent of mucosal injury in different experimental models of colitis in rats and mice[12,13], and also that it increases the mechanical stability of colonic anastomosis in rats[14]. KGF induces proliferation of colonic epithelium (or alternatively decreased apoptosis) and quantitatively increases the mucin content[12,14].

Anastomotic breaking strength or bursting pressure and anastomotic healing in the early postoperative period have been related to collagen synthesis by some authors[29], but not by others[23]. In a porcine model of epidermal wound healing, KGF increased the deposition of collagen fibres in the dermis[9]. In the present study, the results of connective tissue staining show, as previously demonstrated for full-length KGF[14], that tKGF favourably affects the rate of early anastomotic healing through mechanisms other than enhancement of collagen synthesis. KGF may mediate restitution of colonic epithelium and benefit anastomotic healing through its potent properties on cell migration and proliferation of epithelial cells[7,9,13,30–32]. Increased proliferation rate of colonic epithelium in a rat model of colitis has been demonstrated with BrdU staining methods[12]. Further evidence of the proliferative properties of KGF on colonic epithelium is provided by the present study with the finding of significantly increased colonic crypt depth and BrdU-stained cells at each postoperative time point in tKGF-treated animals compared with vehicle-treated animals.

Intra-ileal administration of IGF-I has been shown to have trophic effects on the intestine[17] and IGF-I receptors have been characterized throughout the gastrointestinal tract in several species[18]. Furthermore, it has been demonstrated that IGF-I increases anastomotic collagen content early after operation[22]. The present study also found increased collagen staining after IGF-I administration, but there was a more impressive proliferative response with significantly increased colonic crypt depth and BrdU-stained cells at each postoperative time point. Based on these findings it appears that IGF-I promotes healing of colonic anastomoses by inducing matrix synthesis but also mainly by its proliferative properties on colonic epithelium.

Colonic crypt depth was increased in vehicle animals on postoperative days 4 and 6, and had returned to the values found on day 2 by day 8. An increase of proliferating cells (BrdU staining) was observed on days 2, 4 and 6. It seems that injury-induced and systemically released endogenous growth factors or other reparative mediators induce mucosal proliferation in the colon, not only at the anastomotic sites. This hypothesis is also strengthened by comparison of colonic crypt depth measurement and BrdU staining of mucosal cells from the main study with the results from the background study. After anastomotic surgery, increased colonic crypt depth and BrdU-positive cells were found in comparison with values in unoperated control animals.

KGF has been demonstrated to increase the number of goblet cells as well as mucin production throughout the gastrointestinal tract of rats and mice[7,12,13]. The present study has again shown a marked increase of the number of goblet cells producing acidic mucins after tKGF treatment. However, this is the first time that an increase of the number of goblet cells producing acidic mucins has been demonstrated after IGF-I treatment. This finding also correlates with the increased bursting pressure and reduced inflammatory activity of anastomoses during the same time period in growth factor-treated animals compared with controls. It may be postulated that, through this alteration of mucin glycoprotein content, tKGF as well as IGF-I may restrict the detrimental effects of invasive bacterial organisms and/or injuries that are induced by toxic metabolites[33], hence minimizing the inflammatory reactions and enhancing the healing process. The potential protective effect of mucus is suggested by the finding of a reduced inflammatory reaction at the anastomotic wound site during the first week after surgical intervention in tKGF- and IGF-I-treated animals compared with vehicle-treated animals.

These studies demonstrate for the first time that administration of tKGF as well as IGF-I significantly promotes and accelerates the healing of colonic anastomosis. However, the combined administration of both growth factors does not confer additional benefit. The mechanism(s) by which these growth factors mediate these effects is unknown; however, both tKGF and IGF-I may be acting by accelerating host reparative processes as well as by enhancing protection of the anastomotic wound bed from noxious luminal pathogens. In addition, IGF-I may also be acting by enhancing matrix synthesis.

These findings may provide insight into new potential therapeutic approaches to promote and accelerate healing of colonic anastomosis. Morbidity and mortality associated with anastomotic leaks may be minimized, especially in compromised hosts such as patients with colitis or diabetes and in those needing long-term steroid therapy. It will also be interesting to evaluate the healing of colonic anastomoses by local administration of these growth factors. In the future, it is possible that the traditional use of temporary diverting stomas following operations such as coloanal anastomosis or ileal pouch–anal anastomosis may no longer be needed if growth factors are shown to enhance anastomotic healing even in immunocompromised hosts.

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Short note

Blinded prospective study of the incidence of deep venous thrombosis following conventional or laparoscopic colorectal resection

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Introduction

Reduced venous return from the legs and increased pressure in the inferior vena cava occur during laparoscopic abdominal surgery. This may lead to an increased risk of postoperative deep venous thrombosis (DVT). In a duplex sonographic study, Patel *et al.*[1] detected postoperative DVT in 11 of 20 patients following laparoscopic cholecystectomy. Because of this alarming incidence of DVT, a prospective venous duplex ultrasonographic evaluation of consecutive patients undergoing elective laparoscopic or conventional colorectal resection was performed.

Materials and methods

It was planned to recruit 120 consecutive patients (60 in each group). Laparoscopic resection was attempted for every patient with a body mass index under 32 kg/m^2. Contraindications to laparoscopic resection were tumours of the lower and middle rectum, metastatic disease, tumour size more than 8 cm in diameter, severe intra-abdominal adhesions and synchronous tumours. As described by Caprini *et al.*[2], the patients' risk for perioperative DVT was assessed as low (0–1 points), medium (2–4 points) or high (more than 4 points), according to clinical data (*Table 1*). Antithrombotic prophylaxis consisted of 0·2 ml subcutaneous low molecular weight heparin (Monoembolex®; Sandoz, Basel, Switzerland) daily as well as intermittent pneumatic sequential compression boots (SCD®; Kendall, Neustadt, Germany) at operation.

Before operation and on the fifth postoperative day, duplex ultrasonography was performed by an investigator blinded to the operative technique. Sonographic criteria for thrombosis included pathological vessel compression, reduced vessel expansion during Valsalva's manoeuvre and vein dilatation cranial to the presumed thrombus[3]. Because the incidence of sonographically detected DVT was much lower than expected, an interim analysis was performed after 80 patients. The study was interrupted after 84 patients. Hanley's formula was used to estimate the maximum risk of an adverse event that has not been observed in a finite number of patients[4]. For $n > 30$ the upper limit of the 95 per cent confidence interval for the risk of an event that is compatible with n observations of non-occurrence is calculated by the simple formula $3/n$.

Results

Before operation the groups were well matched clinically and no patient had a coagulation disorder. No patient had a DVT on preoperative ultrasonography. The duration of surgery was about 60 min longer in the laparoscopic group (*Table 1*). Postoperative duplex ultrasonography revealed three DVTs (6 per cent) in the posterior group of calf veins after conventional sigmoid resection but none in the laparoscopic group. The postoperative recovery of patients with a DVT was uneventful; repeat duplex imaging before discharge from hospital showed no sign of ascending thrombosis or floating thrombus in the leg veins. Applying Hanley's formula, the maximum risk of a DVT occurring in the laparoscopic group ($n = 32$) after laparoscopic colorectal resection was estimated to be 9 (95 per cent confidence interval 0–9) per cent.

Discussion

Haemodynamic changes and a prolonged operating time may lead to an increased risk of DVT following laparoscopic abdominal surgery. However, the present results did not confirm the high incidence of thrombosis noted by Patel *et al.*[1] in patients who underwent laparoscopic abdominal surgery. Moreover, the upper 95 per cent confidence interval limit for DVT following laparoscopic colorectal

Table 1 Clinical details of colorectal resections

	Laparoscopic ($n = 32$)	Conventional ($n = 52$)
Age (years)*	64(13)	66(16)
Sex (M : F)	19 : 13	30 : 22
Body mass index (kg/m^2)*	26·7(4·3)	30·5(7·4)
Duration of operation (min)*	232(43)	173(37)
Deep venous thrombosis	0	3
Caprini score		
Low risk	21	38
Medium risk	8	11
High risk	3	3
Indication for operation		
Benign	16	14
Malignant	16	38

*Values are mean(s.d.)

resection calculated for the present data was 9 per cent. These results are comparable to those of Lord *et al.*[5], who detected two DVTs sonographically in 59 patients who underwent laparoscopic cholecystectomy. The risk of DVT following laparoscopic colorectal resection is no higher than 9 per cent when early mobilization, intraoperative pulmonary compression and perioperative low molecular weight heparin are used.

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Value and cost of follow-up after adjuvant treatment of patients with Dukes' C colonic cancer

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Background: The clinical value and costs of different diagnostic tools used to identify potentially curable recurrent disease in patients treated adjuvantly for curatively resected Dukes' C colonic cancer were examined.

Methods: The study group comprised 496 patients treated with chemotherapy over a 1-year interval. Follow-up consisted of interim history, physical examination, liver ultrasonography or computed tomography (CT), measurement of carcinoembryonic antigen (CEA) levels, chest radiography and colonoscopy.

Results: Two hundred and thirteen patients had recurrent disease (median follow-up 43 months). Forty-two patients with recurrence (20 per cent) were treated with curative intent (median survival 38 months; 5-year survival rate 40 per cent). Recurrence was identified by liver ultrasonography or CT ($n = 14$), evaluation of symptoms ($n = 12$), colonoscopy ($n = 8$), CEA measurement ($n = 3$), chest radiography ($n = 2$), physical examination ($n = 1$) and other modalities in two patients. The mean cost of diagnostic procedures per curative resected recurrence for patients amenable to salvage surgery was US$9011. Of all treatable recurrences, 12 of 42 were identified by evaluation of symptoms only. Ultrasonography and colonoscopy identified 22 recurrences at a cost of US$11 790 per patient, while routine follow-up by CEA measurement, chest radiography and physical examination identified a further six at a cost of US$19 850 per patient.

Conclusion: Potentially curable recurrences were detected primarily by liver imaging and colonoscopy. The yield of CEA measurement, chest radiography and physical examination was relatively low; such methods were expensive and should not be recommended in the routine follow-up of these patients.

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Introduction

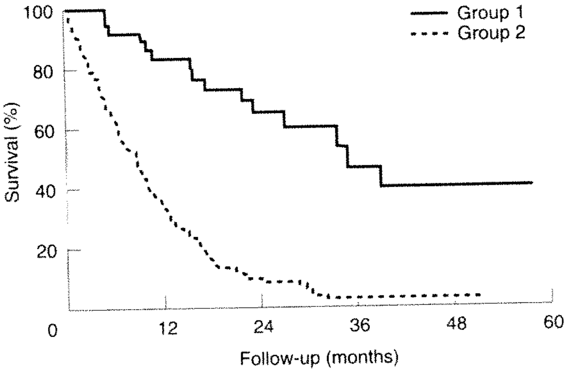
Cost analyses of multiple diagnostic options used to identify potentially resectable recurrences after curatively resected colonic cancer are rare[1–4]. Since contemporary treatment with standard adjuvant chemotherapy in patients with Dukes' C colonic cancer might change future recurrence patterns, up to date information is needed to determine the optimum follow-up regimen in this group. Many studies have investigated the use of carcinoembryonic antigen (CEA) testing even with second-look operation and radio-immunoguided surgery[5–8]. Other studies have concluded that CEA measurement has only a marginal effect on survival and should not be used routinely in the follow-up of patients with colonic cancer[9–11]. The majority of potentially curable recurrent tumours are detected by routine ultrasonography or computed tomography (CT) of the liver while CEA levels are still normal[11–13].

Less controversial is the use of colonoscopy in the detection of local recurrences or metachronous disease[12,13]. Metachronous tumours are mainly located in the left colon and can be examined easily by routine colonoscopy[14–17]. Routine physician visits and simple clinical examination are useful only in the evaluation of symptoms. They are costly and fail to identify resectable recurrences[2]. Most studies have involved various groups of patients with colonic and rectal cancer in a variety of tumour stages, which has made it difficult to make a comparison of studies to determine a

Table 1 Site of first recurrence

	Frequency
Bone	4 (1·9)
Brain	3 (1·4)
Liver	79 (37·1)
Local recurrence	54 (25·4)
Lung	8 (3·8)
More than one site	42 (19·7)
Other	5 (2·3)
Peripheral lymph nodes	13 (6·1)
Peritonitis carcinomatosa	5 (2·3)
Total	213 (100)

Values in parentheses are percentages



No. at risk						
Group 1	171	45	10	2	0	0
Group 2	42	28	12	8	3	2

Fig. 1 Survival curve of patients with recurrent colonic cancer who were eligible for salvage surgery (group 1) and without curative options (group 2). $P < 0{\cdot}0001$ (log rank test)

balanced follow-up programme for patients with Dukes' C colonic cancer. Some studies contain a standard follow-up protocol while in others the follow-up is determined at the discretion of the treating physician[1,3]. Only a few studies contain data relating to patients treated with adjuvant chemotherapy. Because of a possible change in metastatic patterns after chemotherapy older studies on follow-up might be outdated[1–3,10]. To address these issues, the follow-up data of patients with Dukes' C colonic cancer who were treated randomly with adjuvant chemotherapy were studied. The study focused on detection rates and medical costs of physical examination, liver ultrasonography or CT, colonoscopy, chest radiography and CEA tests in identifying patients suitable for salvage surgery.

Patients and methods

Clinical material

In 1991 a randomized multicentre trial was started to compare the effects of 5-fluorouracil–levamisole with leucovorin–5-fluorouracil–levamisole in adjuvant treatment of patients with Dukes' C colonic cancer. This trial was closed in December 1997 and included 500 patients. The clinical results of the trial have been published elsewhere[18]. Follow-up of all patients was standardized according to the protocol rules and was comparable to the study design of Moertel *et al.*[19,20] with the addition of liver ultrasonography or CT at every follow-up visit. Briefly, follow-up consisted of interim history taking and physical examination, liver ultrasonography and CEA tests every 3 months for the first year, every 6 months for the second and third years, and yearly thereafter. CEA levels of 10 ng/ml or above were regarded as abnormal. Chest radiography was performed after 6 and 12 months and yearly thereafter over a period of 5 years. Colonoscopy or barium enema was performed after 2 and 5 years. Median follow-up at analysis was 43 (range 18–96) months. Follow-up for the present analysis was closed in July 1999.

Costs of each diagnostic modality were determined by the 1998 index pricing of the Dutch Ministry of Health (Centrale Overheids Tarief Groep). Each radiological, histological, cytological and/or clinical evidence of recurrent disease was defined as a relapse. Treatable local recurrences included genuine luminal recurrences and limited extraluminal tumour ingrowth at the site of the anastomosis suitable for *en bloc* resection with normal surrounding tissues. Patients were classified into a group without evidence of recurrent disease, a group with recurrence not amenable to curative surgery and a group with recurrent disease accessible for salvage surgery.

Statistical analysis

Overall survival data were illustrated using Kaplan–Meier curves and differences were analysed using the log rank test. The starting point for these calculations was the time of randomization. The relationships between diagnostic modality and salvage surgery were determined by the χ^2 test or Fisher's exact test. Differences were considered to be significant at $P < 0{\cdot}05$.

Results

Four patients were considered ineligible because the disease was more advanced than allowed by the protocol guidelines, leaving 496 patients for analysis. Two hundred and eighty-three patients were alive without evidence of disease and 213 patients developed recurrent disease during follow-up. The

Table 2 Type of diagnostic instrument first able to identify recurrent tumour, leading to salvage surgery

	No resection	Curative resection	Unknown	Total	Diagnostic interventions	Total cost ($)	Cost per curative recurrence ($)
Symptoms	37	12	0	49	—	—	—
CEA measurement	37	3	0	40	1950	15 600	5 200
Chest radiography	4	2	1	7	1025	43 050	21 525
Colonoscopy	5	8	0	13	336	119 616	14 952
Ultrasonography/CT	71	14	2	87	1878/77	139 763	9 983
Combination	7	2	0	9	—	—	—
Physical examination	3	1	0	4	2418	60 450	60 450
Unknown	2	0	2	4	—	—	—
Total	166	42	5	213		378 479	9 011

CEA, carcinoembryonic antigen; CT, computed tomography

sites of first recurrence are presented in *Table 1*. No differences in recurrence were found between the two treatment groups. Forty-two patients (19·7 per cent) underwent salvage surgery with curative intent for recurrent disease with a 5-year survival rate of 40 per cent and a median survival of 38 months (*Fig. 1*). One hundred and seventy-one patients not amenable for curative surgery had a 5-year survival rate of 3 per cent and a median survival of 9 months.

The diagnostic modality that demonstrated first signs of recurrent disease is presented in *Table 2*. Liver ultrasonography or CT demonstrated recurrent metastatic disease most frequently (87 patients; 41 per cent) which led to salvage surgery, usually hepatic resection which was performed in 14 patients. Although colonoscopy demonstrated primary recurrences less frequently, salvage surgery for 'local recurrence' detected by endoscopic examination was possible in eight of 13 patients (seven with genuine suture line recurrence, one with limited extraluminal tumour ingrowth). Resection was also possible in another patient presented with a metachronous tumour located close to the anastomosis. CEA testing showed signs of recurrent disease in 40 patients (19 per cent), which led to salvage surgery in only three. Forty-nine patients (23 per cent) presented with symptoms indicative of recurrent disease. Evaluation of these symptoms coincided with routine visits in five patients, but in the majority they were evaluated between routine visits, which led to salvage surgery in 12 patients. In six patients metastatic tumour at the laparotomy wound or stoma site was locally resectable. Six other patients with an abdominal recurrence, three after right and three after left hemicolectomy, underwent a laparotomy with resection of recurrent tumour. Recurrences in two of these patients were metastases limited to the ovaries. Four patients without symptoms were found to have recurrent disease (two with palpable lymph nodes and two with an abdominal tumour) on routine physical examination. Salvage surgery was possible in only one of these patients.

Table 2 shows the total costs incurred in identifying the 42 patients amenable to salvage surgery. The mean total cost of diagnostic follow-up was $763 per patient (total $378 479 for 496 patients). Cost analysis showed that ultrasonography or CT, CEA testing and evaluation of symptoms identified most primary recurrences. Ultrasonography or CT and colonoscopy together identified more than half of the potentially curable recurrences. Although the proportion of patients with salvage surgery for recurrent cancer identified by the evaluation of symptoms was high (12 of 42 patients), it was insufficient to identify common treatable recurrences. Thus, 34 of the 42 treatable recurrences were identified by ultrasonography or CT, colonoscopy and evaluation of symptoms. Chest radiography, CEA testing and routine physical examination had a low yield and were thus relatively expensive. *Table 3* shows the median time to diagnosis in patients suitable for salvage surgery. Almost all treatable recurrences were identified within 24 months. Only pulmonary metastases were identified after a longer period.

Discussion

Identification of patients with recurrent disease amenable to curative surgery should be the primary goal of follow-up protocols. The largest yield of these recurrences by such a protocol in colonic cancer will be gained in a group of patients with a high proportion of treatable recurrences (*Table 4*)[1–5,21]. Patients with Dukes' C colonic cancer usually have a recurrence rate of 40–50 per cent, while approximately 30 per cent of patients with Dukes' B and 10 per cent of those with Dukes' A cancer will develop recurrent disease. Therefore, it is doubtful whether such follow-up protocols are useful in patients with lymph node-negative tumours[22]. However, patients with these tumours present with similar patterns of treatment failure after potentially curative resection. If effective follow-up modalities were to emerge for patients with Dukes' C cancer it is likely that they would equally useful for detection of recurrence in those with stage B cancers.

In the spectrum of recurrent disease, locoregional recurrences frequently represent incurable disease with generalized abdominal tumour deposits. Most resectable local recurrences are genuine luminal recurrences at the site of the anastomosis. However, the majority of luminal recurrences are in fact extraluminal with tumour ingrowth and are generally incurable. In contrast, distant metastases, particularly to the liver, are often asymptomatic for a long period, but they offer a good chance for salvage surgery. Although most recurrences are identified within 2 years after the initial surgical procedure, lung metastases suitable for resection in this study were identified after follow-up of 24 months. Despite recently published reviews and lack of randomized trials, follow-up programmes consisting of colonoscopy, CEA testing, routine history taking and physical examination are recommended by the American Society of Clinical Oncology[1,2,5,12,23–27]. Liver imaging was not recommended because most studies supporting the use of liver ultrasonography or CT were unable to show curable metastatic lesions before other tests. In the present study, one-third of the patients suitable for salvage surgery were first identified by ultrasonography or CT of the liver. This relatively high detection rate might be the result of a uniform study population and the possible effect of chemotherapy on the recurrence pattern. The combination of diagnostic instruments in this study, including ultrasonography or CT, yielded a total of 42 (20 per cent) of 213 recurrences amenable to salvage therapy. This rate of curable recurrence was substantial in patients who presented with intercurrent symptoms (12 of 42).

Table 3 Median time to detection of recurrent disease in patients amenable for salvage surgery

	No. of patients	Median time (months)
Symptoms	12	12·8
CEA measurement	3	16·2
Chest radiography	2	27·1
Colonoscopy	8	15·2
Ultrasonography or CT	14	14·3
Combination	2	15·3
Physical examination	1	3·5
Total	42	14·7

CEA, carcinoembryonic antigen; CT, computed tomography

The overall survival of patients with a curatively resected recurrent tumour was modest, with a 5-year survival rate of 40 per cent. Minimal or no follow-up would not have identified 30 of 42 patients amenable for salvage surgery before symptoms were present. These results show that multiple diagnostic modalities rather than a single diagnostic instrument are likely to be required to identify the majority of patients with treatable recurrent disease. Routine physical examination, CEA measurement and chest radiography identified only six of 42 such patients. The low detection rate and absolute survival benefit of routine chest radiography (*Tables 1* and *2*) is in accordance with the literature[27,28]. It is not surprising that chest radiography has such a poor yield of detection of treatable recurrence as the identification of pulmonary tumour deposits is invariably associated with a serious systemic disease burden. Safi and Beyer[28] noted that only 15 of 350 recurrences were detected by yearly chest radiography. Seven patients underwent resection of the pulmonary metastases of which only two ultimately were free of disease. The question remains of whether it is worthwhile identifying such patients by these relatively costly diagnostic modalities or whether one should wait for the occurrence of symptoms.

The cost of a single serum CEA test ($8) was low in this study but its ability to identify treatable recurrences was disappointing (three of 40), at a cost of $5200 per salvage

Table 4 Recurrences, curative operations and survival rates in several published studies

Reference	Year	Stage	No. of recurrences	No. of intended curative operations	5-year survival (%)
Ohlsson *et al.*[5]	1995	—	35 of 107 (32·7)	8 of 35 (23)	67
Makela *et al.*[21]	1995	—	22 of 52 (42)	22	57
Audisio *et al.*[4]	1996	I–III	141 of 505 (27·9)	32 of 141 (22·7)	—
Peethambaram *et al.*[3]	1997	II–III	97 of 312 (31·1)	16 of 97 (16)	—
Graham *et al.*[2]	1998	II–III	421 of 1356 (31·0)	96 of 421 (22·8)	—
Goldberg *et al.*[1]	1998	II–III	548 of 1247 (43·9)	222 of 548 (40·5)	23
Present study	2000	III	213 of 496 (42·9)	42 of 213 (19·7)	40

Values in parentheses are percentages

operation. This is in contrast to the findings of Graham *et al.*[2], who concluded that CEA testing was the most cost-effective diagnostic instrument (31 per cent) in identifying treatable recurrences. However, approximately 30 per cent of colorectal carcinomas do not produce CEA and therefore false-negative results are frequently encountered, particularly in poorly differentiated tumours. This might be why CEA testing in patients with Dukes' C colonic cancer may not be worthwhile[21,28,29]. Moreover, CEA level seems to be correlated with tumour volume. A steep rise in CEA concentration usually indicates hepatic metastases not amenable for curative resection. On the other hand, Makela *et al.*[21] showed that CEA testing was superior to other methods in detecting recurrences. In their study ultrasonography was able to detect only four of 22 recurrences before other tests. The present study does not provide a thorough explanation for the higher rate of detection of recurrent tumour by ultrasonography or CT (40·8 per cent; 87 of 213). Although the number of studies that have looked for potentially curable hepatic metastases is relatively small, a meta-analysis showed a survival benefit only in those strategies that included CEA testing[23]. In the present study routine ultrasonography and colonoscopy cost approximately $1000 per recurrence but had a considerable yield (52 per cent) in identifying potential candidates for salvage surgery. Patients who presented with intercurrent symptoms added 28 per cent (12 of 42) of treatable recurrences to those identified with ultrasonography and colonoscopy.

It is concluded that multiple diagnostic modalities are likely to be required to identify most patients with treatable recurrent disease after curative resection and adjuvant treatment of Dukes' C colonic cancer. Liver imaging (ultrasonography or CT) and colonoscopy can identify most treatable recurrences. The yield of routine CEA testing, chest radiography and routine physician visits is low at relatively high cost.

Acknowledgements

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Original article

High-risk colorectal adenomas and serum insulin-like growth factors

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Background: This study investigated the hypothesis that circulating levels of insulin-like growth factor (IGF) I and its main binding protein (IGFBP-3) predict for the presence of colorectal adenomas, surrogate markers of colorectal cancer risk.

Methods: Within the Flexi-Scope Trial (healthy volunteers aged 55–64 years), at one study centre, IGF-I and IGFBP-3 levels in serum samples collected prospectively from 442 attendants were measured. Of these, 100 individuals underwent a complete screening colonoscopy. There were 47 normal examinations, while in 11 examinations low-risk adenomas and in 42 examinations high-risk adenomas were identified. Estimates of relative risk (RR) for the adenomatous stages were calculated by means of unconditional logistic regression, adjusting for known risk factors.

Results: Mean serum IGF-I and IGFBP-3 levels were similar in individuals with a normal colonoscopy finding and in those with low-risk adenomas. By contrast, the mean(s.d.) serum IGF-I level was increased (190(53) *versus* 169(54) µg/l; $P = 0.06$) and the serum IGFBP-3 concentration was significantly decreased (3·22(0·60) *versus* 3·47(0·62) mg/l; $P = 0.05$) in individuals with high-risk adenomas compared with levels in those with normal colonoscopy and low-risk adenomas combined. Levels were unaffected by removal of the adenomas. With high-risk adenoma as the dependent factor, regression models demonstrated a significant positive association with IGF-I after controlling for IGFBP-3 (RR per one standard deviation (1s.d.) change 4·39 (95 per cent confidence interval (c.i.) 1·31–14·7); $P = 0.02$) and, independently, an inverse association with IGFBP-3 after adjustment for IGF-I (RR per 1s.d. change 0·41 (95 per cent c.i. 0·20–0·82); $P = 0.01$).

Conclusion: These findings suggest that circulating IGF-I and IGFBP-3 levels are related to future colorectal cancer risk and, specifically, may predict adenoma progression.

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Introduction

Insulin-like growth factor (IGF) I is a growth hormone (GH)-dependent regulatory peptide which is both mitogenic and antiapoptotic, attributes that may favour tumour development[1]. In the circulation, IGF-I is bound predominantly (more than 90 per cent) to the major insulin-like growth factor binding protein, IGFBP-3. Circulating IGFBP-3 concentration is also GH dependent, and both IGF-I and IGFBP-3 levels are age dependent, declining steadily with advancing years after puberty[2]. Across the general population there are wide interindividual variations and it is postulated that this variability in physiology may affect cancer risk[3,4]. This has been borne out in epidemiological studies which have shown a strong positive association between circulating IGF-I levels, and, independently, an inverse association with IGFBP-3 concentration, and the subsequent risk of developing breast, prostate and colorectal cancer[5–7].

Evidence for a potential role of IGF-I in colorectal carcinogenesis derives from its ability to influence the proliferative behaviour of normal colonic epithelium and colonic cancer cell lines[8,9]. Further evidence is gleaned from the observation that acromegaly, an endocrine disorder characterized by sustained raised levels of GH and IGF-I, is associated with an increased risk of developing colonic cancer[10] and a tendency towards more advanced adenomas[11]. The aim of the present study was to examine further the role of circulating IGF-I and IGFBP-3 in colorectal carcinogenesis, specifically to correlate serum levels of these growth factors with the different stages of adenoma development, from early 'low risk' adenomas through to advanced 'high risk' adenomas.

Patients and methods

The Flexi-Scope Trial

The Flexi-Scope Trial is a multicentre randomized trial to evaluate the effect of a 'once only' flexible sigmoidoscopy at age 55–64 years in the prevention of colorectal cancer morbidity and mortality[12,13]. Persons with previous colonic pathology or who had a large bowel endoscopy within the previous 2 years were excluded at baseline. Participants randomized to the screening arm of the trial were offered a flexible sigmoidoscopic examination. The trial protocol dictated that all polyps were removed and/or biopsied, and examined histologically. Polyps were categorized as hyperplastic or adenomatous; the latter were further classified as tubular, tubulovillous or villous. Epithelial dysplasia was graded as mild, moderate or severe in accordance with World Health Organization criteria[14]. Individuals with adenomas were subclassified into low or high risk, the latter defined where at least one adenoma was 1 cm or more in diameter, of tubulovillous or villous morphology, demonstrated severe dysplasia and/or where three or more adenomas were present[15]. All individuals with high-risk adenomas subsequently underwent a complete colonoscopic examination. In addition to the main trial, screening with intentional complete colonoscopy was undertaken within a substudy of individuals with a family history of colorectal cancer (two or more first-degree relatives) and compared with volunteer individuals (controls) without a family history.

Sample for analysis

At one study centre (Christie Hospital NHS Trust), baseline blood samples were collected prospectively from 442 individuals before they underwent diagnostic endoscopy. To define the risk for all colorectal adenomas precisely, this analysis specifically examined only those individuals ($n = 100$; 60 men and 40 women) who underwent a complete screening colonoscopy to the caecum (*Fig. 1*). All colonoscopies were performed by a single endoscopist (J.E.P.). A second blood sample was taken 6–8 weeks after colonoscopy from 18 individuals with a normal examination, and from a further 18 patients with high-risk adenomas 6–8 weeks postpolypectomy. Height and weight were measured in all participants, and data were recorded on colorectal cancer risk including family history, aspirin use, cigarette smoking and use of hormonal replacement therapy (HRT). The study was approved by the local ethics committee for South Manchester Health Authorities.

Assays

Serum IGF-I concentration was measured, following acid–alcohol extraction, by a well established radioimmunoassay using a polyclonal rabbit antiserum (R557A) raised against purified human IGF-I[16]. The intra-assay coefficient of variation (CV) for mean IGF-I concentrations of 46, 246 and 706 µg/l was 11·3, 6·5 and 4·7 per cent respectively. The sensitivity of this assay was 14 µg/l. IGFBP-3 concentration was measured by means of an immunoradiometric assay with a commercially available reagent (Diagnostic Systems Laboratories, Webster, Texas, USA). The intra-assay CV for mean serum concentrations of 1·0, 2·2 and 9·8 mg/l was 6·1, 4·1 and 4·4 per cent respectively. The sensitivity of this assay was 0·5 mg/l. All samples were assayed in triplicate and laboratory staff were blinded to the status of adenoma or control.

Statistical analysis

As IGF-I and IGFBP-3 determinants were normally distributed, parametric statistics were applied and data presented as mean(s.d.). The molar ratios of IGF-I to IGFBP-3 were also assessed as an indicator of IGF-I bioavailability (for conversion, 1 µg/l is 0·130 nmol/l for IGF-I and 0·036 nmol/l for IGFBP-3)[2]. Differences between means were compared with the Student *t* test (paired and unpaired) and one-way analysis of variance as

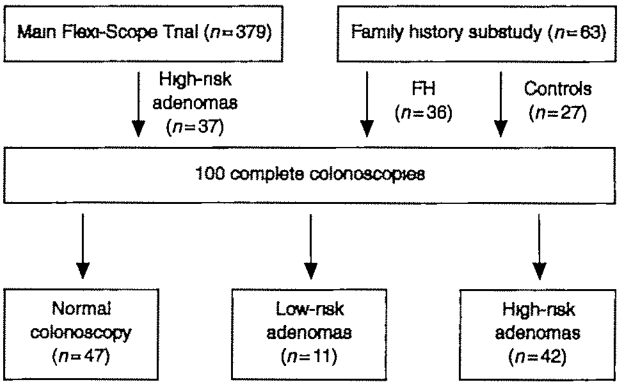
Main Flexi-Scope Trial (n=379)
Family history substudy (n=63)
High-risk adenomas (n=37)
FH (n=36)
Controls (n=27)
100 complete colonoscopies
Normal colonoscopy (n=47)
Low-risk adenomas (n=11)
High-risk adenomas (n=42)

Fig. 1 The family history substudy compared participants with a strong family history of colorectal cancer (FH; two or more first-degree relatives) with participants without a family history who volunteered to have a complete colonoscopy (controls). Both groups were initially randomized in accordance with the main trial criteria

appropriate. Correlations were expressed as Pearson coefficients (r) and categorial data were compared with the χ^2 test.

To test for trends, IGF-I and IGFBP-3 levels were used as continuous variables in unconditional logistic regression models. Because IGFBP-3 levels were correlated with IGF-I ($r = 0{\cdot}59$, $P = 0{\cdot}01$) and may have opposite effects on risk, it was necessary to adjust for these factors simultaneously in the models to assess their independent effects. As there is evidence that a single measurement of IGF-I and IGFBP-3 concentration is representative of longer-term levels[5], odds ratios in the models were taken as estimates of relative risk (RR). All tests were two tailed; $P < 0{\cdot}05$ was considered to indicate statistical significance. SPSS 9.0® (Superior Performing Software Systems, Chicago, Illinois, USA) and SPlus® (MathSoft, Seattle, Washington, USA) computer software was used for computations.

Results

There were 42 individuals with high-risk adenomas, 11 with low-risk adenomas, and 47 with a normal colonoscopy finding. Thirty individuals, 24 of whom also had adenomas (19 high risk), had hyperplastic polyps.

Serum levels of insulin-like growth factor I and insulin-like growth factor I binding protein

Mean IGF-I and IGFBP-3 levels, and IGF-I–IGFBP-3 molar ratios for those with normal colonoscopy findings, low-risk and high-risk adenomas are shown in *Table 1*. There were striking similarities between values for IGF-I, IGFBP-3 and the molar ratios for individuals with normal colonoscopy findings and low-risk adenomas. By contrast, the mean(s.d.) serum IGF-I concentration was raised, although not significantly so (190(53) *versus* 169(54) µg/l; $P = 0{\cdot}06$), and the serum IGFBP-3 level was significantly lower in individuals with high-risk adenomas (3·22(0·60) *versus* 3·47(0·62) mg/l; $P = 0{\cdot}05$) compared with low-risk adenomas and normal colonoscopy combined. Expressed as a molar ratio, the mean IGF-I–IGFBP-3 value was significantly higher in individuals with a high-risk adenoma than in those with low-risk adenoma and normal colonoscopy findings taken together ($P = 0{\cdot}002$). Differences in IGF-I, IGFBP-3 and molar ratios between individuals with and without hyperplastic polyps were tested, but no distinctive patterns were found (data not shown).

Repeat samples

To assess for possible influences on serum IGF-I and IGFBP-3 levels due to the presence of an adenoma in an individual, measurements were performed on samples taken before and after adenoma removal. In 18 individuals with high-risk adenomas, no differences were found before and after polypectomy in serum IGF-I levels (mean(s.d.) 188(80) *versus* 193(74) µg/l; $P = 0{\cdot}47$, paired t test) and IGFBP-3 concentration (3·26(0·67) *versus* 3·36(0·47) mg/l; $P = 0{\cdot}36$). Similarly, in 18 individuals with a normal colonoscopy finding, there were no differences in mean(s.d.) serum IGF-I concentration (173(80) *versus* 169(74) µg/l; $P = 0{\cdot}74$) and IGFBP-3 level (3·56(0·55) *versus* 3·47(0·59) mg/l; $P = 0{\cdot}56$) in samples taken before and after colonoscopy. For all 36 repeat samples there was a strong correlation between the two time-points for both IGF-I ($r = 0{\cdot}74$, $P < 0{\cdot}001$) and IGFBP-3 ($r = 0{\cdot}82$, $P < 0{\cdot}001$) concentrations.

Predictive models

Based on the initial observations, the predictability of serum IGF-I and IGFBP-3 concentration for the presence of high-risk adenomas as the dependent variable was assessed using multiple logistic regression models. All models included adjustments for known colorectal cancer risk factors including age, sex, current use of HRT, body mass index (body-weight in kg/(height in m)^2), daily aspirin use and cigarette smoking (never smoked *versus* ex-smokers and current smokers).

The first model tested the predictability of IGF-I concentration (*Table 2*). Tested alone (unadjusted for

Table 1 Serum insulin-like growth factor-I, insulin-like growth factor binding protein-3 and combined molar ratios in the total cohort

	Normal colonoscopy ($n = 47$)	Low-risk adenomas ($n = 11$)	High-risk adenomas ($n = 42$)	P*	P†
IGF-I (µg/l)	168(56)	169(50)	190(53)	0·15	0·06
IGFBP-3 (mg/l)	3·46(0·62)	3·49(0·63)	3·22(0·60)	0·14	0·05
IGF-I–IGFBP-3 molar ratio	0·22(0·08)	0·22(0·07)	0·26(0·06)	0·009	0·002

Values are mean(s.d.). IGF, insulin-like growth factor; IGFBP, insulin-like growth factor binding protein. *Comparison between the three groups (one-way analysis of variance); †high-risk adenoma *versus* the other two groups (Student t test comparison of independent means)

Table 2 Regression analysis for the ability of insulin-like growth factor-I to predict high-risk adenomas

	Unadjusted for IGFBP-3		Adjusted for IGFBP-3*	
	RR per 1s.d. change	P	RR per 1s.d. change	P
IGF-I	1·30 (0·80–2·11)	0·28	4·39 (1·31–14·7)	0·02
Age			1·04 (0·88–1·25)	0·60
Sex (men as referent)			0·40 (0·15–1·09)	0·07
Smoking (never smoked as referent)			1·49 (0·52–4·29)	0·45
Body mass index			1·15 (1·01–1·31)	0·03
Aspirin (non-user as referent)			0·39 (0·91–1·78)	0·23
Family history†			3·18 (0·19–51·9)	0·41

Values in parentheses are 95 per cent confidence intervals. For high-risk adenoma group *versus* others: age (mean 60·7 *versus* 60·2 years; P not significant, t test); males (32 of 42 *versus* 28 of 58; $P = 0{\cdot}005$, χ^2 test); smoking (ten of 42 *versus* 13 of 58; P not significant, χ^2 test); body mass index (mean(s.d.) 26·8(2·8) *versus* 25·9(4·2) kg/m^2; P not significant, t test); aspirin usage (three of 42 *versus* ten of 58; P not significant, χ^2 test); family history (four of seven *versus* 33 of 56; P not significant, χ^2 test). *Insulin-like growth factor (IGF)-I and insulin-like growth factor binding protein (IGFBP)-3 were treated as interactive terms. †Model limited to family history substudy ($n = 63$) on an 'intention to treat' basis; by intent, participants with a strong family history and controls underwent a complete screening colonoscopy. RR, relative risk; 1s.d., one standard deviation

Table 3 Regression analysis for the ability of insulin-like growth factor binding protein-3 to predict high-risk adenomas

	Unadjusted for IGF-I		Adjusted for IGF-I*	
	RR per 1s.d. change	P	RR per 1s.d. change	P
IGFBP-3	0·47 (0·17–1·02)	0·07	0·41 (0·20–0·82)	0·01
Age			1·05 (0·88–1·24)	0·62
Sex (men as referent)			0·39 (0·14–1·04)	0·06
Smoking (never smoked as referent)			1·29 (0·51–4·16)	0·48
Body mass index			1·16 (1·02–1·32)	0·03
Aspirin (non-user as referent)			0·40 (0·09–1·77)	0·23
Family history†			3·72 (0·19–71·5)	0·38

Values in parentheses are 95 per cent confidence intervals. *Insulin-like growth factor (IGF)-I and insulin-like growth factor binding protein (IGFBP)-3 were treated as interactive terms. †Model limited to family history substudy ($n = 63$) on an 'intention to treat' basis; by intent, participants with a strong family history and controls underwent a complete screening colonoscopy. RR, relative risk; 1s.d., one standard deviation

IGFBP-3 concentration), there was no association between IGF-I levels and the presence of high-risk adenomas. However, controlling for IGFBP-3 in this model revealed a positive association between IGF-I and the dependent variable: an increase in IGF-I level of 56 µg/l (one standard deviation (1s.d.) in individuals with no adenoma) corresponded to more than a fourfold increase in risk (RR 4·39 (95 per cent confidence interval (c.i.) 1·31–14·7)). To test for the influence of family history, the analysis was repeated limiting the model to the 63 patients in the family history substudy; the positive association between IGF-I concentration and the presence of high-risk adenomas remained.

In the second model the predictive characteristics of IGFBP-3 concentration were tested (*Table 3*). In the absence of controlling for IGF-I, there was a non-significant inverse association between IGFBP-3 and the presence of high-risk adenomas. After adjustment for IGF-I level, the model revealed that IGFBP-3 concentration was independently and inversely related to the presence of high-risk adenoma: an increase in IGFBP-3 level of 1s.d. (0·62 mg/l) corresponded to a 59 per cent decrease in risk (RR 0·41 (95 per cent c.i. 0·20–0·82)). Again, limiting the model to those in the family history substudy, the significant inverse association with IGFBP-3 concentration remained.

Discussion

This study has shown a positive association between serum IGF-I concentration and, independently, an inverse association between serum IGFBP-3 concentration and the presence of high-risk adenomas (surrogate markers of colorectal cancer risk)[5]. These observations support epidemiological evidence that the IGF-I–IGFBP-3 profile may predict an individual's cancer risk[5–7]. In addition, the study has suggested that circulating IGF-I and IGFBP-3 levels may predict tumour progression from early through to more advanced adenomas.

The findings of the present study substantiate the observations on the risk of colorectal cancer in men (Physicians Health Study)[7] and a recently reported study on the risk of late-stage adenoma in women (Nurses Health Study)[17]. In particular, the definition of 'late stage' adenoma in the latter equates with that of 'high risk' used in the present study. While these epidemiological studies had greater statistical power than the present study, and had the advantage that blood samples were collected many years before tumour presentation, cases were identified by participant reporting and hence there were potential ascertainment biases. The present study was smaller and cross-sectional in design but had the advantage that controls were defined by colonoscopic findings in screened healthy subjects. Other strengths were an age-defined population, prospective blood collection and diagnosis using uniform histological criteria.

The present study has shown that serum IGF-I and IGFBP-3 levels are constant over the intermediate term and unaffected by the presence of adenoma. This is in contrast to serum IGF-II and IGFBP-2 levels, which are raised in individuals with colorectal adenomas and normalize after polypectomy[18]. Taken together with the above-mentioned epidemiological studies, the characteristics of serum IGF-I and IGFBP-3 levels are best described as predictive (rather than tumour markers) and may be compared with other described risk factors for colorectal cancer. Thus, the risk associated with a 1 s.d. change in serum IGF-I was similar to that for individuals with a strong family history of colorectal cancer[19]. However, as the risk due to family history represents a relatively small contribution to overall colorectal cancer demographics, the potential predictive characteristics of serum IGF-I concentration become more relevant to the overall population. On the other hand, the negative association of IGFBP-3 concentration was of a similar order to the protective effect for regular aspirin usage. Thus, for instance, aspirin users with upper tertile IGFBP-3 values had a significantly lower proportion of high-risk adenomas than non-users with lower tertile IGFBP-3 values (two of seven *versus* 18 of 28; $P = 0.02$).

The authors have previously reported no association between IGF-I and IGFBP-3 levels and the presence of (all) adenomas in a study limited to screening with flexible sigmoidoscopy[20]. In that report only 19 of the total 56 individuals with adenomas were high risk, and no statistical differences emerged. It appears that serum IGF-I and IGFBP-3 levels in individuals with low-risk adenomas and with a normal colonoscopy finding are the same. Thereafter, the advance from early to late adenoma occurs in only a small proportion of all adenomas (best estimate is probably less than 10 per cent)[21] and a hypothesis emerges that an individual's IGF-I–IGFBP-3 profile may predict this progression. A number of biological mechanisms make this hypothesis plausible. IGF-I is both mitogenic and antiapoptotic via the IGF-I receptor and, in turn, IGF-I receptors are universally expressed in colorectal adenomas and adenocarcinomas (unpublished observations). The importance of IGF-I to tumour progression is exemplified by the demonstration of inhibited tumour growth by blockade of the IGF-I receptor[22]. On the other hand, IGFBP-3 was originally considered as a binding protein regulating the interaction of IGF-I and its receptor, but more recently has been appreciated as a potent proapoptotic agent independent of the IGF-I receptor[23].

There is indirect epidemiological evidence supporting a role for circulating IGF-I–IGFBP-3 in colorectal carcinogenesis. Holly *et al.*[3] have pointed out that the GH–IGF-I axis has a pivotal role in regulating somatic longitudinal growth and that final adult height correlates with the incidence of colorectal tumours[24]. These studies imply that the risk of cancer may be established from early life and that factors such as nutrition in childhood may have a 'programming' effect via the GH–IGF-I axis. Contrary to this, the present data and the Nurses Health study[17] suggest that IGF-I and IGFBP-3 levels predict adenoma progression. As adenomas are uncommon in the general population before the age of 40 years, the relative amounts of IGF-I and IGFBP-3 in mid adult life may be more relevant. This may also explain why in acromegaly, an endocrine disorder characterized by very high sustained serum IGF-I levels, there is only a modest increase in colorectal cancer incidence with no evidence of premature presentation, despite a peak incidence for the diagnosis of acromegaly in early adulthood[10]. Additional indirect associations include: (1) higher mean IGF-I concentration – lower mean IGFBP-3 levels[25] in males may explain the male predominance in colorectal cancer incidence; (2) orally administered oestrogen lowers IGF-I concentration[26] and the use of HRT in postmenopausal women is protective against colorectal cancer[27]; and (3) long-term cigarette smoking lowers serum IGFBP-3 concentration[25] and is positively associated with colorectal adenoma[28].

In conclusion, this study supports the view that circulating levels of IGF-I and IGFBP-3 are related to future colorectal cancer risk[4]. Specifically, the IGF-I–IGFBP-3 profile may be an important determinant of adenoma progression. If substantiated, these findings may be relevant to clinical assessment of risk. Furthermore, these data provide an 'endocrine' hypothesis for colorectal tumour development, and a new perspective in our understanding of colorectal carcinogenesis.

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Original article

Prospective evaluation of the morbidity of axillary clearance for breast cancer

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Background: Axillary clearance, despite its morbidity, retains an essential role in the management of patients with breast cancer. The aim of this prospective study was to document the development of arm swelling and limitation of shoulder movement following complete axillary clearance.

Methods: One hundred patients who had axillary clearance to level III, for treatment of breast cancer, were followed prospectively for over 1 year. Arm volumes were measured using an optoelectronic volometer and shoulder movements with a goniometer.

Results: Ten patients had significant arm swelling at 1 year. The swelling was mild in eight and moderate in two. No patient developed severe swelling. Reduced arm movements were noted in the first week after operation but had returned to normal at 6 months.

Conclusion: This study provided accurate documentation of the morbidity associated with axillary clearance, together with a reproducible method of arm volume measurement.

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Introduction

Axillary node involvement is recognized as the single most important factor in the staging of carcinoma of the breast[1]. Clinical assessment of the axilla is highly inaccurate[2] and axillary sampling can frequently underestimate the extent of nodal disease[1,3,4]. While recent reports on the intraoperative detection of sentinel lymph nodes[3–8] are promising, axillary clearance remains the most accurate means of determining lymph node status. Axillary clearance is associated with lower levels of local recurrence than other forms of management, but at the expense of significant morbidity, in particular the limitation of shoulder function and arm swelling[9–11].

The measurement of changes in arm volume has been difficult to perform in an accurate and reproducible manner. Arm circumference, measured with a tape measure at set points from a bony prominence, is convenient and has been used frequently, but it is inaccurate[12,13]. The measurement of limb volume by water displacement is accurate if performed by experienced personnel on a mobile patient, but is difficult, time consuming and unsuitable for use immediately after surgery. The volometer is an efficient and accurate method of performing these measurements.

The aim of this study was to document the development of arm swelling and limitation in shoulder movement in patients who had a mastectomy or breast-conserving surgery in association with axillary clearance for unilateral breast cancer.

Patients and methods

One hundred patients with unilateral breast cancer were followed prospectively from the time of surgery for 1–2 years. All patients had axillary clearance to level III, together with mastectomy or wide local excision. All surgery was performed in one surgical unit as described previously[14] and was continuously audited for quality control[15]. No intravenous access was acquired in the side undergoing axillary dissection. The need for adjuvant therapy was assessed individually but no patient received axillary radiotherapy. All patients were seen before and after operation by the physiotherapist and instructed in exercises to be performed after operation to maximize the range of shoulder movement.

All arm volume measurements were carried out by a single operator using an optoelectronic volometer (German Bösl Medizintechnik, Hamburg, Germany). Significant

Table 1 Number of patients with mild or moderate swelling at 6 months, 1 and 2 years after operation

	No. of patients	Mild swelling (200–400 ml)	Moderate swelling (> 400 ml)
6 months	89	4 (4)	0 (0)
1 year	100	8 (8)	2 (2)
2 years	42	6 (14)	2 (5)

Values in parentheses are percentages

swelling was taken as a relative increase in the volume of the ipsilateral arm of more than 200 ml[16]. Patients were assessed before operation and then twice after surgery (days 2 and 5). Follow-up measurements were taken at 6 months, 1 and 2 years after operation.

The range of movement of both shoulders was assessed with a goniometer, a hand-held instrument for the measurement of angles. The maximum range of flexion, extension, abduction, adduction and external rotation was measured. Values were compared with those on the contralateral side and the preoperative range. A subjective assessment of morbidity was carried out using a questionnaire. When swelling was detected patients were referred for physiotherapy with exercise, massage and compression bandaging.

Volometry

The volometer is an optoelectronic measuring device[13]. It consists of a square sensor frame into which are set 480 infrared-emitting photoconductor diodes and receptors. The frame is set on rollers and the arm is positioned horizontally and perpendicular to the plane of light. The arm is measured for 36 cm from the distal end of the radius. A measurement of the cross-sectional area of the arm is made every 2 mm and used to calculate a value for total arm volume. Each arm volume measurement was based on the mean of three separate measurements.

To test the reproducibility of the volometer, repeated measurements were taken of a single arm, replacing the arm after each reading. The standard deviation after 30 measurements was 0·5 per cent (8 ml). If the arm was left in position between measurements the standard deviation was 0·2 per cent. In addition, the volometer readings were found to correlate well with plethysmographic measurements (interclass correlation coefficient 0·96) and gave more precise results than the corresponding measures of water displacement (average deviation from the mean 3·8 *versus* 10·7 ml for water displacement). These results are similar to those produced by Fischbach *et al.*[13].

Questionnaire

At each follow-up visit the patients were asked a set of questions to evaluate the subjective evidence of morbidity. Changes in arm volume were graded as none, mild, moderate and severe. Mild swelling, corresponding to measured changes of between 200 and 400 ml, is not immediately apparent to others, is often intermittent and does not interfere with the use of the arm. With moderate swelling the difference between the arms is clearly visible, although it can still be disguised by clothing. When swelling is severe, usually corresponding to a measured difference of more than 700 ml, arm function is diminished constituting a major disability for the patient. Limitation of shoulder movement was graded similarly according to its functional significance.

Results

One hundred patients were followed prospectively to at least 1 year after operation. The mean age of the patients was 53 (range 30–79) years. Sixty-four patients underwent mastectomy and 36 breast-conserving surgery in association with an axillary clearance. The mean(s.d.) number of nodes excised was 27(9). Forty-five patients had nodal metastases; among these the mean number of positive nodes was 7.

All patients were assessed before operation, 90 in the immediate postoperative period (days 2 and 5), 89 at 6 months and 100 at 1 year. To date, 42 patients have been assessed 2 years after surgery. The data from this group have been included in some of the analysis.

Arm volumes

In the perioperative period there was no significant change in the mean volume of the ipsilateral arm relative to the other side. No patient developed significant arm swelling during this time.

After 6 months, four patients had significant arm swelling (*Table 1*). In all cases the measured difference was less than 300 ml. When these four patients were followed to 1 year the measured difference in arm volume was found to have improved in all patients, and in two it had dropped below the level of significance.

Of the 100 patients seen at 1 year, ten had swelling of the ipsilateral arm of more than 200 ml. In eight the swelling was mild (less than 400 ml) and did not cause significant disfigurement or disability. In two the measured difference was over 400 ml, constituting moderate swelling (*Table 1*). No patient had a measured difference of more than 700 ml, or a degree of swelling that interfered with normal activities.

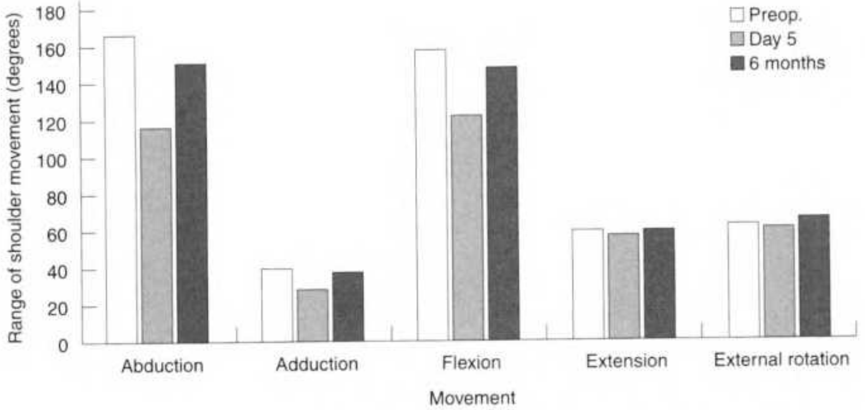

Fig. 1 Range of shoulder movement of ipsilateral arm at 5 days and 6 months after operation compared with preoperative value

Of the eight patients with arm swelling, seven have been assessed at 2 years so far. In three the swelling had resolved, in two it remained unchanged and in two it had progressed.

There was no association between the development of arm swelling and the type of breast surgery performed (mastectomy *versus* breast-conserving surgery), the number of nodes retrieved or the extent of axillary node involvement. Forty-five patients developed a postoperative seroma, one of whom had arm swelling at 1 year. Likewise only one patient with a postoperative wound infection subsequently developed arm swelling. While none of these factors was shown to increase the risk of lymphoedema, the number of patients involved was too small to draw significant conclusions.

Arm movements

Arm movements were expressed as the range of shoulder movement in degrees. Considerable reduction in the range of movement, especially in flexion, abduction and adduction, was observed in the immediate postoperative period (*Fig. 1*). However, this improved considerably with time. Within 6 months of surgery, the majority of the patients regained over 90 per cent of the initial range of movement. A significant reduction of movement resulting in interference with lifestyle or activities was found in only two patients at 6 months.

Discussion

Axillary clearance is performed routinely in the treatment of early breast cancer in this unit. The benefits are that it allows complete staging of the disease, it theoretically eliminates the possibility of future axillary recurrence and it may produce a marginal improvement in survival[17]. These benefits must be weighed against the potential complications of the operation, in particular reduced shoulder movement and arm swelling.

The incidence of arm swelling has been reported recently at between 6 and 30 per cent[16,18–23]. The reported incidence varies with the method used to define significant swelling, the source of the patients, the completeness of follow-up, and the interval between axillary treatment and limb measurement. In the 1960s, when radical mastectomy and modified radical mastectomy were the only treatment options, the incidence of swelling also varied greatly. In a review by American authors Britton and Nelson[24] the incidence in nine reports varied between 6·7 and 62·5 per cent. A similar review by British authors Hughes and Patel[25] found a range of 41–70 per cent.

In the present study the incidence of arm swelling at 12 months' follow-up was 10 per cent. Although the follow-up was short, the changes in arm volume were evaluated accurately in a group of patients who all received extensive axillary surgery (mean lymph node retrieval 27 nodes) without axillary radiotherapy. While it is likely that the incidence of swelling will increase with longer follow-up, it is noteworthy that in patients who had arm swelling detected at an early stage (measured difference 200–300 ml), time and physiotherapy led to resolution of the swelling. The potential value of the volometer may be in the detection of minor changes in arm volume which will allow the initiation of treatment at a stage before the swelling becomes obvious clinically.

In the present study, volume measurements were carried out using an optoelectronic volometer. The volometer offers a number of advantages over the alternatives of tape measure or water displacement. It gives accurate and reproducible results. It is easy to use and each measurement takes only a few seconds, allowing multiple measurements to be performed at each visit and a mean to be calculated.

With the current interest in sentinel node biopsy as an alternative form of axillary management in breast cancer, the role of axillary clearance is under review. The present study provides accurate documentation of the morbidity associated with this procedure, and describes a method to standardize the collection of morbidity data. This will enable more meaningful comparison to be made between alternative methods of management of the axilla.

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Detection, treatment and outcome of axillary recurrence after axillary clearance for invasive breast cancer

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Background: The aim was to gain insight into the diagnosis, treatment and prognosis of axillary recurrence after axillary clearance for invasive breast cancer in a large patient series.

Methods: Between 1984 and 1994, 4669 patients with invasive breast cancer underwent axillary clearance in eight community hospitals in the south-eastern part of the Netherlands. Using follow-up data in a population-based cancer registry, 59 patients with axillary recurrence were identified.

Results: The median interval between treatment of the primary tumour and the diagnosis of axillary recurrence was 2·6 (range 0·3–10·7) years. In 51 patients (86 per cent), axillary recurrence was found by palpation during routine follow-up. Surgery was part of the treatment of recurrence for 41 of 59 patients. Regional control (complete eradication of axillary recurrence) was achieved in 34 patients (58 per cent). The 5-year actuarial survival rate was 39 (95 per cent confidence interval 25–53) per cent. Patients with negative axillary lymph nodes at the time of diagnosis of the primary tumour and complete eradication of axillary recurrence had the best prognosis.

Conclusion: Patients with axillary recurrence had a poor prognosis, except when complete eradication was achieved and axillary lymph nodes were negative at the time of diagnosis of the primary tumour.

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Introduction

The objectives of axillary clearance in patients with invasive breast cancer include staging of the disease and prevention of regional recurrence. Less invasive techniques have been introduced recently; sentinel node biopsy is the most promising[1]. At present, most patients with invasive breast cancer still undergo axillary clearance. In most series, the cumulative risk of axillary recurrence (AR) after full axillary clearance or dissection of the lower and middle parts of the axilla (levels I and II) varies from 0·5 to 3·0 per cent[2–10].

To gain insight into the diagnosis, treatment and prognosis of AR requires study of large patient series. Most studies on AR have been based on fewer than 30 patients. Furthermore, axillary failure is often not listed separately from other nodal sites of failure. Using follow-up data in a population-based cancer registry, 59 patients were identified with AR after axillary clearance in the treatment of invasive breast cancer. Diagnosis, treatment and treatment outcome were studied, and determinants of a favourable outcome after AR were sought.

Patients and methods

Patient data were retrieved from the population-based Eindhoven Cancer Registry, which serves a population of almost one million inhabitants in the south-eastern part of the Netherlands (6 per cent of the Dutch population). Data had been collected by the cancer registry from copies of the pathology reports of ten pathologists from three different laboratories and the medical records of eight community hospitals and one department of radiotherapy. During the interval 1984–1994, 4669 (86 per cent) of the 5408 patients with invasive breast cancer underwent axillary clearance. Two hundred and eight patients (4 per cent) had either concurrent or sequential bilateral carcinomas, resulting in a total of 4877 axillary dissections.

In the treatment guidelines of the regional Breast Cancer Study Group, the borders of axillary dissection are the latissimus dorsi muscle (dorsal), the thoracic wall below the major and minor pectoral muscles (ventral) and the lower border of the axillary vein (cranial). Axillary clearance was not always considered necessary for primary tumours measuring 0·5 cm or less and was usually omitted for inoperable cancer and for the very elderly. The mean number of lymph nodes examined by the pathologist after axillary clearance was ten. Fewer than six nodes were reported in 14 per cent and more than 15 in 12 per cent of the operations.

Irradiation of the axilla and the supraclavicular field was recommended for patients with inadequate axillary dissection, extracapsular extension of tumour growth or nodal involvement in the apex of the axilla. The axillary field was usually exposed to 52·5 Gy (25 fractions) and the supraclavicular field to 40 Gy (20 fractions). The prescribed doses were specified at 30 mm below the skin surface. Adjuvant systemic treatment was recommended only for axillary node-positive patients. Chemotherapy was indicated for premenopausal node-positive patients and endocrine therapy (tamoxifen) for postmenopausal node-positive patients with an oestrogen receptor-positive tumour.

Table 1 Characteristics at the time of diagnosis of the primary tumour in 59 patients who later developed axillary recurrence

	No. of patients
Age at diagnosis of primary tumour (years)	
<50	18 (31)
50–69	27 (46)
≥70	14 (24)
Histological type of primary tumour	
Ductal	47 (80)
Lobular/mixed	10 (17)
Other	2 (3)
Surgery for primary tumour	
Mastectomy	33 (56)
Breast-conserving treatment	24 (41)
Other	2 (3)
Size of primary tumour (mm)	
≤20	25 (42)
>20	34 (58)
Number of lymph nodes in axillary specimen	
≤5	18 (31)
6–15	32 (54)
>16	7 (12)
Unknown	2 (3)
No. of positive lymph nodes in axillary specimen	
0	32 (54)
1–3	14 (24)
>3	11 (19)
Unknown	2 (3)
Radiotherapy for primary tumour	
Breast or chest wall only	25 (42)
Breast or chest wall and axillary or supraclavicular field	7 (12)
No	27 (46)
Adjuvant systemic treatment for primary tumour	
Chemotherapy	3 (5)
Hormonal therapy	15 (25)
None	41 (69)

Values in parentheses are percentages

Following treatment patients were seen alternately by the radiation oncologist and the referring surgeon. They were usually reviewed every 3 months during the first 2 years, every 6 months during the following 3 years and yearly thereafter. Bone scans, chest radiography, complete blood count and liver function tests were not used routinely, but were arranged for signs and symptoms suggestive of distant disease or after the diagnosis of local or regional recurrence.

Since 1989, the Eindhoven Cancer Registry has recorded follow-up information on all patients with breast cancer, including the date and site of local, regional and distant recurrence, and the date of death. The information is provided by the pathologists, radiotherapists and the majority of surgeons. As of January 1998, 79 patients without metastases at the time of diagnosis were reported to have AR.

After examination of the clinical records, 20 patients were excluded. Eight patients had distant disease at the time of diagnosis of AR, in nine AR was reclassified as supraclavicular disease and for three patients AR could not be confirmed. Thus, 59 patients remained available for analysis; two had a concurrent recurrence in the breast, four an earlier recurrence in the breast and one an earlier recurrence in the chest wall. The medical record of each patient was reviewed to collect detailed information on the diagnosis and treatment of the AR. The primary treatment of the 59 patients took place between 1984 and 1994 and the AR was diagnosed between 1986 and 1998. The mean age of the patients at the time of primary diagnosis of breast cancer was 58 (range 35–90) years. Characteristics of the patients and primary tumours and treatment are presented in *Table 1*.

Statistical analysis

Follow-up was complete until 1 January 1998. Survival analysis was carried out using the life-table method to evaluate the prognosis after AR. Endpoints were overall survival, distant disease-free survival and survival without subsequent locoregional recurrence. Survival curves were calculated from the date of treatment of AR or the date of the diagnosis of AR when treatment was not given. The following variables were analysed to assess their ability to predict overall survival: age at diagnosis of AR, size of the primary tumour, lymph node status of the primary tumour (positive or negative), number of positive lymph nodes, time interval from initial surgery to AR, size of AR, surgical treatment of AR (yes or no), completeness of surgery for AR (microscopically complete or incomplete excision), and response to treatment of AR (complete eradication *versus* stable or progressive disease). Actuarial curves were

Table 2 Treatment of 59 patients with axillary recurrence and achievement of regional control

		Complete eradication of axillary recurrence	
Treatment	No. of patients	Yes	No
S*	5 (8)	2	2
S+RT	11 (19)	9	2
S+RT+CT	2 (3)	2	0
S+RT+HT	15 (25)	10	5
S+RT+CT+HT	1 (2)	0	1
S+CT	1 (2)	1	0
S+HT	6 (10)	4	2
RT	8 (14)	3	5
RT+HT	7 (12)	3	4
CT	1 (2)	0	1
HT	1 (2)	0	1
None	1 (2)	0	1
Total	59 (100)	34 (58)	24 (41)

Values in parentheses are percentages. *Information on regional control was missing for one patient. S, surgery; RT, radiotherapy; CT, chemotherapy; HT, hormonal therapy

compared by means of the two-tailed log rank test. Because of the small number of patients, multivariate analyses were not performed.

Results

Diagnosis

The mean age of the patients at the time of diagnosis of AR was 61 (range 39–99) years. The median interval between treatment of the primary tumour and diagnosis of AR was 2·6 (range 0·3–10·7) years. Of patients with a tumour larger than 20 mm, 20 of 34 developed AR within 2·5 years of treatment compared with eight of 25 patients with smaller tumours ($P = 0{\cdot}04$). Of patients with primary axillary nodal involvement, 17 of 25 developed AR within 2·5 years of treatment compared with ten of 32 of those without ($P = 0{\cdot}006$).

The AR was found during a routine follow-up visit by simple palpation in 51 patients (86 per cent). Thirty-one (53 per cent) had complaints at the time of diagnosis of AR, most commonly swelling and pain, or a stinging feeling in the axilla. The extent of the AR was recorded in only 27 patients (46 per cent).

Treatment

Treatment was curative in 40 cases and palliative in 18 patients. The intent of treatment was unknown for one patient. For 41 of 59 patients, treatment consisted of surgery, with or without adjuvant radiotherapy or systemic therapy (*Table 2*). Seventeen patients did not receive surgical treatment and one patient refused treatment. The excision was microscopically complete in 25 of the 41 patients who had surgery. For two patients, the completeness of the excision was doubtful and in seven patients it was incomplete. No information about the completeness of the resection was available in seven patients. Thirty-four patients received systemic therapy; 29 hormonal therapy (mostly tamoxifen), four chemotherapy and one both chemotherapy and hormonal therapy (*Table 2*).

Regional control (complete eradication of AR) was achieved in 34 patients (58 per cent). Twenty-four patients (41 per cent) showed stable or progressive disease. The response to treatment was not known for one patient (*Table 2*). After treatment, 24 patients (41 per cent) suffered one or more complications according to medical records: 12 patients had oedema of the arm, 15 had pain in the arm or shoulder and three reported problems with arm movements.

Prognosis

The median length of follow-up after diagnosis of AR was 2·8 (range 0·3–9·2) years for the total group and 4·4 (range 0·9–9·2) years for the patients who were still alive. Of the 59 patients with AR, 36 died (61 per cent), 32 after developing distant metastases. Five patients were still alive with distant metastases. Five years after AR, the actuarial overall survival rate was 39 (95 per cent confidence interval (c.i.) 25–53) per cent and the distant recurrence-free survival rate was 35 (95 per cent c.i. 21–50) per cent.

The results of univariate analysis of prognostic variables for survival are shown in *Table 3*. The best prognosis was observed for the subgroup of 22 patients whose AR could be eradicated completely and who had negative axillary lymph nodes at the time of diagnosis of the primary tumour; of these 22 patients only five died. In contrast, all 12 patients with stable or progressive disease after treatment of AR and positive lymph nodes at first presentation died within 2 years of diagnosis of the AR.

Subsequent locoregional recurrence developed in eight of the 34 patients in whom regional control was initially achieved after treatment of AR. At 5 years, the subsequent locoregional recurrence-free survival rate was 70 (95 per cent c.i. 50–90) per cent.

Discussion

The rate of AR found in this study was 1·3 per cent (59/4669). Because follow-up information has only been recorded in the cancer registry since 1989, documentation was incomplete for early AR occurring in patients

Table 3 Univariate analysis of factors related to death for 59 patients with axillary recurrence

	No. of patients	No. of deaths	Median survival (years)*	*P*‡‡
Age at diagnosis of AR (years)				
<50	15	8 (53)	3·4	0·07 (*versus* ≥70 years)
50–69	28	16 (57)	4·8	0·96 (*versus* <50 years)
≥70	16	12 (75)	1·7	0·05 (*versus* 50–69 years)
Time interval to AR (years)				
≤2·5	28	21 (75)	2·4	0·13
>2·5	31	15 (48)	4·8	
Complaints at diagnosis of AR‡				
Yes	31	19 (61)	2·5	0·57
No	18	10 (56)	3·1	
Intent of treatment§				
Curative	40	19 (48)	5·4	<0·001
Palliative	18	16 (89)	1·3	
Extent of AR (mm)¶				
≤20	15	6 (40)	—†	0·35
>20	12	8 (67)	—†	
Surgical treatment of AR				
Yes	41	21 (51)	4·8	0·01
No	18	15 (83)	1·9	
Completeness of surgery for AR**				
Microscopically complete	25	9 (36)	6·8	0·05
Microscopically incomplete	9	6 (67)	1·5	
Response to treatment§				
Complete eradication of AR	34	13 (38)	1·5	<0·001
Incomplete eradication of AR or progressive disease	24	22 (92)	6·8	
Size of the primary tumour (mm)				
≤20	25	10 (40)	5·4	0·005
>20	34	26 (76)	1·8	
Lymph node status of the primary tumour††				
Negative	32	13 (41)	5·4	<0·001
Positive	25	21 (87)	1·6	
No. of positive lymph nodes at diagnosis of primary tumour††				
0	32	13 (41)	5·4	<0·001 (*versus* >3)
1–3	14	10 (71)	2·9	0·04 (*versus* 0)
>3	11	11 (100)	1·2	0·01 (*versus* 1–3)

Values in parentheses are percentages. *Estimated time since treatment of axillary recurrence (AR) during which 50 per cent of patients died; †median survival was not reached; ‡ten unknown; §one unknown; ¶32 unknown; **four unknown; ††two unknown; ‡‡log rank test

diagnosed between 1984 and 1988. These patients constituted 40 per cent of the 4669 patients in the study interval, so the real rate of AR was estimated to be approximately 2·5 per cent. This percentage remains within the 0·5–3·0 per cent range after complete or level I and II axillary clearance reported in other studies, mostly of stage I or II breast cancer[2–10].

The risk of AR after axillary clearance should be the gold standard with which to compare the effectiveness of more limited approaches to the axillary lymph nodes. Sentinel lymph node biopsy seems to be the most promising alternative[1,11]. The risk of AR after sentinel node biopsy should not be higher than the risk after axillary dissection. It remains unclear whether equal regional control rates can be obtained after sentinel node biopsy as follow-up of most series is still very short.

The prognosis in patients with AR is poor. Comparison with other studies is complicated owing to the small number of patients in most series. In a study of 13 patients with AR, four died after a median follow-up of 8 years[2]. Renolleau *et al.*[6] described the course of isolated AR in 26 patients, nine of whom developed metastases. In a study by Recht *et al.*[4], 11 of 22 patients with AR were alive without evidence of further locoregional or systemic disease at a median follow-up of 45 months. In a study by Fowble *et al.*[9] three of six patients with isolated AR remained alive without evidence of disease 30, 43 and 103 months after salvage therapy.

The present study defined a subset of patients with AR who may be long-term survivors. The best prognosis was found for the subgroup of 22 patients with complete eradication of AR and without axillary nodal involvement at the time of diagnosis of the primary tumour. The important prognostic value of the axillary nodal status of the primary tumour has also been demonstrated in studies of patients with local or locoregional recurrence after breast-conserving treatment or mastectomy[12–15]. There may be several explanations for the better prognosis of patients with negative lymph nodes. One might be the slower growth rate, so that follow-up is still too short. Another could be a better response to systemic treatment. The rapid occurrence of clinical signs of distant disease among the patients with positive lymph nodes at the time of diagnosis of the primary tumour suggests that their AR is an indicator of the presence of substantial metastatic deposits elsewhere, rendering a curative effect of systemic treatment less likely.

Treatment of AR appeared to be associated with serious morbidity in more than 40 per cent of the patients. Considering that this information was collected retrospectively from the notes in the clinical records, the actual morbidity rate is probably higher.

AR remains uncommon among patients with early breast cancer when adequate axillary surgery is performed. Treatment of patients with AR is highly individualized, but prognosis is poor, except when complete eradication of the recurrence can be achieved and axillary lymph nodes were negative at the time of diagnosis of the primary tumour.

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Original article

Impact of mammographic interval on stage and survival after the diagnosis of contralateral breast cancer

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Background: Following treatment for primary breast cancer, annual mammography and regular clinical breast examination is advised. The aim of this study was to investigate whether annual mammography resulted in an improvement in stage of contralateral breast cancers compared with mammography performed at a longer interval.

Methods: This was a retrospective analysis of patients treated for breast cancer, who developed a contralateral breast cancer between 1977 and 1998. Patients were stratified into two groups according to mammographic interval.

Results: Data were available for 275 patients who developed contralateral breast cancer during follow-up. Annual mammography was performed in 51 per cent; these patients were a mean of 5 years younger at diagnosis. Patients who had annual or biennial mammography had comparable rates of impalpable contralateral breast cancer, 30 and 27 per cent respectively. The tumours were of favourable stages in 60 and 58 per cent respectively. Five-year disease-free survival following diagnosis of contralateral breast cancer was 75 per cent in both groups. When the contralateral lesion was detected by mammography, disease-free survival was better, irrespective of the stage of the ipsilateral breast cancer.

Conclusion: No difference was found between the two groups in stage distribution and disease-free survival after the diagnosis of contralateral breast cancer. Survival was better in patients in whom the contralateral breast cancer was first detected by mammography.

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Introduction

In a patient with treated breast cancer, the relative risk of developing a contralateral cancer is two to six times greater than the risk of developing a first breast cancer in the general population[1]. Storm and Jensen[2] estimated this risk to be 25 per cent for women diagnosed with breast cancer at the age of 45 years who survived until the age of 75 years. For metachronous contralateral breast cancer a constant annual incidence of 0·5–1·0 per cent is seen[1,3–5]. The results of population screening for breast cancer (earlier stage, better outcome and mortality reduction[6]) justify screening patients who survive after treatment of breast cancer. Regular mammography leads to improvement in stage distribution for second cancers[7,8], and is recommended together with clinical breast examination[9–11].

The aim of this study was to compare the outcome of metachronous contralateral breast cancer detected during follow-up using different mammographic intervals. The results were compared with those of the Dutch population screening programme.

Patients and methods

Between January 1976 and December 1990, approximately 3900 patients treated for stage 0, I, II and IIIA breast cancer were listed in the tumour registry of the Netherlands Cancer Institute/Antoni van Leeuwenhoek Ziekenhuis (NKI/AvL). Five hundred and eighty-one patients were registered with bilateral breast cancer. The intake and review of patients with contralateral breast cancer was terminated in December 1997; follow-up was available until December 1998. The case files from these patients were reviewed.

Only patients with metachronous contralateral breast cancer were included in the study. Metachronous was defined as the diagnosis of a second breast cancer at least 1 year after the first. The main selection criteria for this study included surgery and/or radiotherapy for both breast cancers and follow-up at the NKI/AvL. Patients with lobular *in situ* carcinoma were excluded because this lesion is not considered malignant; also excluded were patients with distant disease at diagnosis of the metachronous contralateral lesion. Patients with a local recurrence treated within 2 years before the diagnosis of the contralateral breast cancer were excluded because of the possibility of metastases in the contralateral breast. These exclusions accounted for 258 (44 per cent) of the patients. One file was missing.

A total of 323 contralateral malignancies was available for study. The following data were retrieved from the case files: age, dates of diagnosis and stages of both breast cancers, number of clinical breast examinations and number of mammograms performed following diagnosis of the first breast cancer, results of the mammography series, method of first detection, last follow-up date or time and cause of death.

In the NKI/AvL, patients treated for primary breast cancer are routinely followed with breast examination every 3 months for the first 2 years, every 6 months for the subsequent 3 years and then annually until death. During 1977–1985 follow-up mammography was not performed routinely. After 1984, when new equipment was installed, gradually more patients had annual mammography. Usually in patients over 60 years of age mammography was performed at longer intervals. For the study, two groups with different mammographic intervals were analysed. The first group consisted of all women with a mean mammographic interval of 1 year, following presently accepted guidelines. With regard to inevitable variations in appointment dates the cesura was chosen at 15 months. The second group included all patients with a longer mean mammographic interval (15 months or longer).

The method of detection was defined as the way in which the metachronous contralateral breast cancer was first detected, either clinical examination or mammography. Detection by clinical examination was defined as the situation in which the patient or physician found a sign of breast cancer at any time during follow-up. This included when clinical signs were found around the time of a scheduled mammogram, but the result was not yet known. Mammography was the method of detection when the patient was contacted for further investigation because of a suspicious lesion, after a non-suspicious follow-up visit. Mammography was also the method of first detection when the suspicious result of the mammogram was known to the physician before clinical examination. Four radiologists read the mammography series. The original reports were used for this study.

The breast cancer treatment protocol remained generally consistent during the study period. In patients with clinically operable T_2 and T_3 contralateral breast tumours a preoperative axillary apex biopsy was done[12]. Locoregional treatment in patients with a positive apex node consisted of radiotherapy to the breast and regional lymph node basins, when distant metastases were excluded. All patients otherwise operable underwent either a modified radical mastectomy or breast-conserving treatment with full axillary clearance. Forty-eight per cent of the patients with operable breast cancer in both breasts had a bilateral modified radical mastectomy. Breast-conserving therapy for both breasts was performed in 31 per cent.

The internationally accepted Union Internacional Contra la Cancrum (UICC) staging classification was used[13]. Histological type, tumour size and node status were found in the original pathology reports. When tumour size was not recorded, the slides (when available) were reviewed.

Data on follow-up and mammography series were complete for 275 (85 per cent) of the remaining 323 eligible patients with metachronous contralateral breast cancer. Data were incomplete in three patients because of lack of compliance to the follow-up scheme. In 45 patients, the mode of detection was uncertain because of partial follow-up elsewhere.

Statistical methods are described in the relevant parts of the results and the tables.

Results

Group 1 consisted of 141 patients (51 per cent) who had annual mammography with a mean interval of 12 months and a mean total number of 5·7 mammograms per patient. The 134 patients in group 2 had a mean interval of 23 months and a mean total number of 3·4 mammograms per patient.

Patient and tumour characteristics are shown in *Tables 1* and 2. The patients in group 1 were 3 and 5 years younger at diagnosis of the ipsilateral and contralateral breast cancers respectively than those in group 2. No differences were found in the other variables: impalpable disease, first detection with mammography, interval between both breast cancers, stage and survival.

Forty per cent of the contralateral breast cancers were first suspected at mammography, slightly more in group 2 than in group 1: 44 *versus* 35 per cent ($P = 0{\cdot}15$, χ^2 test). For the impalpable contralateral breast cancers detected with mammography, there was no difference in age between the groups with different mammographic intervals: 62 years ($n = 33$) *versus* 61 years ($n = 34$) ($P = 0{\cdot}67$, two-sample t test).

The mammography results of 185 patients at diagnosis of both breast cancers were reviewed. In the remaining 90 patients, results of the first breast cancer were not known because the mammogram was not performed or was done elsewhere. When the ipsilateral breast cancer was visible on the mammogram, the chance of detecting a malignancy in the second breast with mammography was 81 per cent. This chance was 49 per cent when the first breast cancer was not visible ($P < 0{\cdot}0001$, χ^2 test). Adjusting for the mammographic results of the contralateral breast cancers, patients with a negative mammogram of the ipsilateral breast cancer were a mean of 8 (s.e. 2) years younger than patients with a positive mammogram of the ipsilateral lesion ($P = 0{\cdot}0001$, analysis of variance (ANOVA)). Patients with a negative mammogram of the contralateral breast were 4(2) years younger when adjusted for the ipsilateral mammographic result ($P = 0{\cdot}030$, ANOVA). Both effects seemed to be additive ($P = 0{\cdot}84$, ANOVA test for interaction).

There was no difference in disease-specific survival between the two groups with varying frequency of mammography ($P = 1{\cdot}00$) (*Table 2*). Patients with contralateral breast cancer detected by mammography died less frequently from breast cancer (15 per cent at 5 years) than those with contralateral breast cancer detected by other means (31 per cent at 5 years) ($P = 0{\cdot}015$) (*Fig. 1*). This cannot be explained by differences in stage distribution of the ipsilateral breast cancer. In a Cox proportional hazard regression model stratified by pathological stage of the ipsilateral breast cancer, the value for method of detection was still $P = 0{\cdot}015$.

Table 3 shows a comparison between stage in palpable and screen-detected breast cancers in the Dutch population, and the present screening results in contralateral breast cancer. Screening the contralateral breast with mammography, clinical breast examination and breast self-examination in breast cancer survivors (aged 27–95 years) produced comparable cancer stage as screening for breast cancer in the Dutch population (aged 50–69 years) with mammography alone[15].

Table 1 Characteristics of patients with metachronous contralateral breast cancer

	All patients (n=275)	Group 1 (n=141)	Group 2 (n=134)	P*
Age at dignosis (years)				
IBC	52(13)	51(12)	54(14)	0·006
CBC	58(13)	56(12)	61(14)	
Interval (months)				
Mammography	17(12)	12(2)	23(15)	
IBC until CBC	73(48)	68(45)	78(49)	0·11

Values are mean(s.e.). Group 1, annual mammograms; group 2, biennial mammograms. IBC, ipsilateral (first) breast cancer; CBC, contralateral (second) breast cancer. *Two-sample test

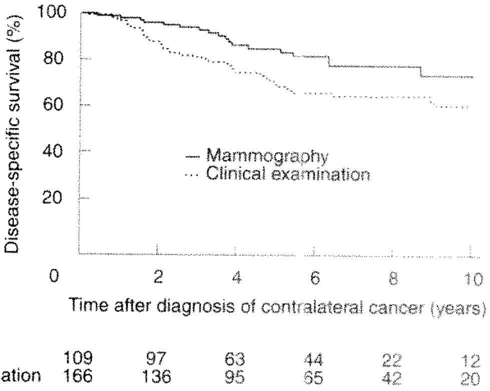

Fig. 1 Disease-specific survival according to method of detection of metachronous contralateral breast cancer, 1977–1998. $\chi^2 = 5{\cdot}98$, $P = 0{\cdot}015$ (log rank test)

Table 2 Tumour characteristics of metachronous contralateral breast cancer and disease-free survival after treatment

	All patients (n=275)	Group 1 (n=141)	Group 2 (n=134)	P
Detected with mammography	109 (40)	50 (35)	59 (44)	0·15‡
Impalpable	78 (28)†	42 (30)	36 (27)†	0·62‡
pN_0	164 of 239 (69)	87 of 126 (69)	77 of 113 (68)	0·88‡
Pathological stage				
0	28 of 232 (12)	15 of 126 (12)	13 of 106 (12)	
I	108 of 232 (47)	60 of 126 (48)	48 of 106 (45)	0·77‡
Survival (%)*	75 (3)	75 (5)	75 (5)	1·00§

Unless otherwise indicated values in parentheses are percentages. Group 1, annual mammograms; group 2, biennial mammograms *Five-year disease-specific survival (with standard error) after contralateral breast cancer. †Data not available for one patient. pN, pathological node stage. ‡χ^2 test; §log rank test

Table 3 Pathological stage in contralateral breast cancer compared to Dutch breast cancer screening programme

				Stage (%)					
Reference	Year	Method of detection	Patient age (years)	pT_{is}	pT_{1a}	pT_{1b}	pT_{1c}	$\geqslant pT_2$	pN_1
Dutch population screening programme									
NETB[6]	1995	Palpation		4	1	6	31	50	
NETB[6]	1995	First screen		13	4	22	39	17	
Fracheboud *et al.*[14]	1998	Subsequent screens	50–69	15	5	24	35	17	24
Peer *et al.*[15]	1994	Screens + interval cancer	40–75	13	22*		42	24	31
Present study	2000	Screens + palpation	27–95	12	4	26	31	26	31

NETB, National Evaluation Team for Breast Cancer Screening. pT, pathological tumour stage; pT_{is}, *in situ* carcinoma; pT_{1a}, tumour 1–5 mm; pT_{1b}, tumour 6–10 mm; pT_{1c}, tumour 11–20 mm; pN_1, node-positive pathological nodal stage. *pT_{1a} and pT_{1b} combined

Discussion

The American Society of Clinical Oncology recommends annual mammography in women with a previous diagnosis of breast cancer[11]. In this retrospective comparison of two groups of survivors after breast cancer who underwent annual mammography or mammography at longer intervals, tumour characteristics and outcome of the contralateral breast cancer were similar. However, women who had annual mammography were a mean of 5 years younger when diagnosed with a second breast cancer. This age difference is a reflection of the practice in the NKI/AvL, where clinicians tended to perform mammography at a longer interval in older women.

The data from this review support an adjustment to the follow-up recommendations concerning mammographic screening of the contralateral breast but, because of the retrospective nature of the study, a recommendation for a 2-year mammographic interval in all patients is debatable. Such an interval might be too long in younger women, because growth rates in breast cancer are age dependent and higher rates have been noted in younger women[16]. The present data suggest that for older patients (aged more than than 60 years) an interval of 2 years is justifiable. This practice does not appear to have led to a worse prognosis for the group with a longer mammographic interval, with respect either to stage or survival.

The quality of the mammograms improved during the study. This is important because it translates into an improved chance of finding signs of malignancy with mammography. In the older population (more than 60 years), in which the overall assessment of mammography is better, a similar proportion of contralateral breast cancers was found by mammography (after 1986): 53 per cent in group 1 and 51 per cent in group 2 (data not shown).

The follow-up protocol included regular clinical breast examination and instructions for breast self-examination, as is generally considered good clinical practice in patients with breast cancer. Population screening does not include these modalities. In population screening programmes, the published interval cancer rates are 18–43 per cent, depending on age and mammographic interval[17–20]. The favourable stages 0 and I are seen in 46–50 per cent[18], or stage I alone in 39–47 per cent[18,20,21].

In this study 40 per cent of the contralateral breast cancers were first detected by mammography. These tumours were diagnosed at an earlier stage and survival was improved, even when accounting for the stage of the first tumour. Sixty per cent of the contralateral tumours should be considered as 'interval cancers' found by the patient or physician; 47 per cent of these clinically detected cancers were in UICC stages 0 or I. One factor that may have biased these results is the fact that before 1985 mammography was not used routinely, so a contralateral breast cancer detected in that period was more often palpable, while the mean interval between mammograms was longer. However, when this was taken into account in the statistical analysis, none of the conclusions stated above were affected (data not shown).

In this retrospective study of screening the contralateral breast with mammography and clinical examination, 59 per cent of the contralateral breast cancers were in the favourable stages 0 and I. This met the quality standards of the mammography audit for population screening (over 50 per cent of breast cancers should be in stages 0 and I[22]). It also met the standard regarding ductal *in situ* cancer and tumours smaller than 11 mm (more than 30 per cent), 42 per cent in the present study. The pathological node (pN) standard of less than 25 per cent with axillary lymph node metastases was not met (in this study 31 per cent). This might have been affected by the NKI/AvL policy to perform a full level three axillary clearance for treatment.

As many patients had a small contralateral breast cancer, they could have been treated with breast-conserving therapy. Many of them preferred a mastectomy, either because the first cancer was treated with mastectomy, or because of fear of a third cancer.

The result of screening the contralateral breast (*Table 3*) measured by the most relevant surrogate endpoints was equivalent to that of the Dutch population screening programme[6,14,15]. The results may be biased, because group 1 contained more younger women, in whom failed mammography interpretation occurs more often[23]. Kerlikowske *et al.*[23] also demonstrated that interpretation more often fails in women with a family history of breast cancer. This could be another bias in this study; 55 per cent of the women younger than 50 years of age had at least one family member with breast cancer.

Screening with a longer mammographic interval in older patients with breast cancer survivors may be justifiable. The chance of detecting a contralateral breast cancer with mammography is higher when the ipsilateral breast cancer was visible on mammography. Patients with a mammographically detected contralateral breast cancer had improved survival compared with patients whose cancer was detected clinically, irrespective of the stage of the ipsilateral breast cancer.

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Original article

Laparoscopically assisted total or distal gastrectomy with lymph node dissection for early gastric cancer

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Background: A secure lymphadenectomy in a laparoscopically assisted gastrectomy performed for gastric cancer is required because of the high prevalence of lymph node metastasis. A surgical technique for laparoscopic gastrectomy with lymph node dissection and reconstruction using a conventional circular stapler is reported.

Methods: Forty-nine laparoscopically assisted gastrectomies with lymphadenectomy (47 distal and two total gastrectomies) were performed using devices for retraction of the stomach and laparoscopic ligation of arteries, which were developed to ensure secure dissection of lymph nodes. Reconstruction by Billroth I or intestinal interposition using a conventional circular stapler was performed through a small incision through which the specimen was removed. When submucosal invasion was suspected ($n = 16$), the lymph nodes along with the common hepatic artery were also dissected through the same incision.

Results: The operations were performed without serious complication. None was converted to laparotomy, and there were no deaths. Metastatic lymph nodes were seen in perigastric nodes and nodes along the left gastric artery in five cases. In five of the 49 patients the macroscopic diagnosis of depth of invasion was underestimated.

Conclusion: A technique of laparoscopic gastrectomy with lymph node dissection for early invasive gastric cancer is described. A definitive answer concerning the appropriate level of lymph node dissection and the role of laparoscopic gastrectomy in the treatment of more advanced gastric cancer remains to be defined.

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Introduction

To date, many laparoscopic devices have been developed and laparoscopic procedures are used widely in many abdominal operations. For gastric malignancies, endoscopic and laparoscopic treatments have been performed, including endoscopic or laparoscopic mucosectomy[1] and partial gastrectomy with a laparoscopic stapler[2–4]. Recently, laparoscopically assisted gastrectomy with perigastric lymph node dissection at an early stage of gastric cancer was reported to improve postoperative quality of life[5–7]. Because gastric cancer, even in the early stages of invasion, tends to metastasize beyond the perigastric lymph nodes, it was necessary to devise an advanced laparoscopic technique that provides a secure and safe method for performing lymphadenectomy, including ligation of gastric and gastroepiploic arteries. This study employed a surgical technique that allows reconstruction with a circular stapler after laparoscopic total or distal gastrectomy with lymph node dissection.

Patients and methods

Patients

From July 1998 to September 1999, 49 patients with gastric cancer, in whom the depth of tumour invasion had been estimated before operation as limited to the mucosa or submucosa, underwent laparoscopically assisted gastrectomy. Informed consent was obtained from all patients. Preoperative staging was based on clinical examination, gastrointestinal endoscopy, endoscopic ultrasonography and upper gastrointestinal contrast radiography. Before operation 0·5 ml carbon (CH-40; Katayama Chemical

Company, Osaka, Japan) suspension was injected endoscopically into the submucosal layer at the oral edge of the lesion to identify the margins for performing the laparoscopic gastrectomy[8]. Of 49 patients, 47 underwent a laparoscopically assisted distal gastrectomy with Billroth I anastomosis using a circular stapler for reconstruction. In two cases a total gastrectomy was performed with reconstruction by intestinal interposition.

Technique for distal gastrectomy

The laparoscopic operation was performed in the supine position under carbon dioxide pneumoperitoneum. Four surgical ports were inserted in the upper abdomen, and a flexible gastrointestinal fibrescope (GE-500HR; Fujinon, Tokyo, Japan) with a 120° viewing angle was employed as a laparoscope to obtain a wide laparoscopic view. Carbon dioxide was inflated through the biopsy channel of the scope. Two retraction tubes placed around the antrum and gastric body were used to lift the stomach to the abdominal wall (*Fig. 1*). A loop wire was connected to one end of the tubes. The loop was used when the tube was inserted into the abdominal cavity through a direct abdominal wall puncture. These retraction tubes allowed a view of the bursa omentalis and eliminated the need for use of laparoscopic forceps and additional surgical ports. The tip of the laparoscope was introduced into the bursa omentalis. The pedicles of the right and left gastric arteries and the right gastroepiploic artery were identified, divided and ligated with a newly developed ligation forceps. The jaw of the forceps has an indentation that traps an already tied knot near one end of a ligation thread. After applying the forceps to the artery, one end of the thread was withdrawn to fix the knot on the other side of the jaw (*Fig. 2a*). After carrying the thread under the artery, the same forceps was used as a knot-pusher during an extracorporeal ligation (*Fig. 2b*).

The lesser omentum, abdominal oesophagus and lesser curvature of the stomach were dissected for lymphadenectomy using coagulating scissors. The greater omentum and left gastroepiploic vessels were ligated in the same manner as for the dissection of lymph nodes along the greater curvature. A transverse laparotomy was performed through a 4-cm skin incision at the epigastrium, and the anvil of a circular stapler (Premium plus CEEA, 31 mm; United States Surgical Cooperation, Norwalk, CT, USA) was inserted through a small incision made in the anterior wall of the pylorus (*Fig. 3a*). The anvil was fixed with a band[9]. After delivery of the distal stomach out of the abdomen, a 45-mm Endo-GIA (United States Surgical Cooperation, Norwalk, CT, USA) was applied to the greater curvature of

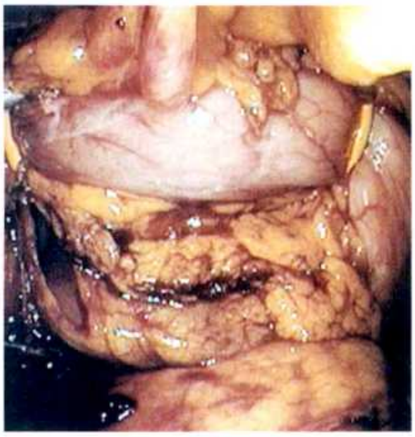
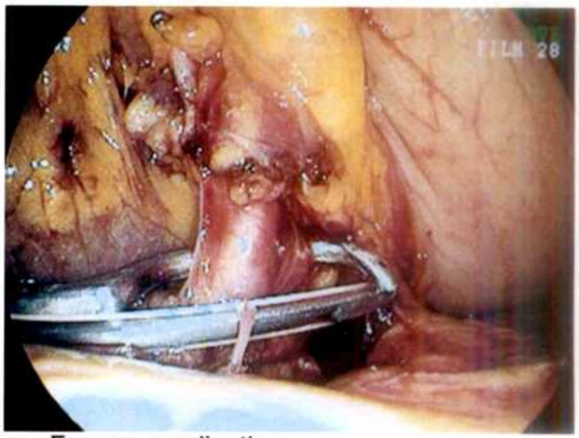
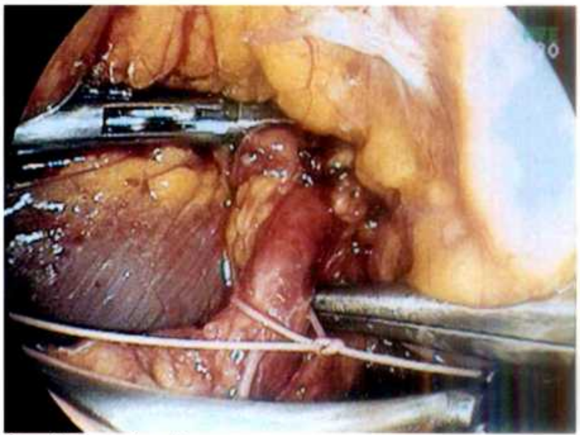


Fig. 1 The stomach lifted and fixed to the abdominal wall with the use of retraction tubes



a **Forceps application**



b **Artery ligation**

Fig. 2 Laparoscopic view during ligation of the right gastric artery after dissection of lymph nodes. **a** The ligation forceps was applied to the right gastric artery to carry the thread under the artery. **b** The artery was ligated by using the same forceps as a knot pusher

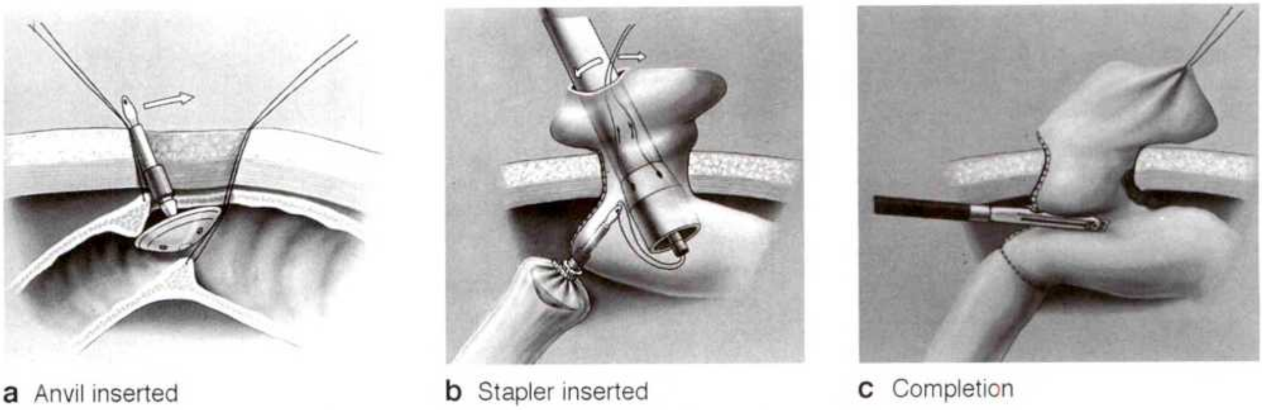

a Anvil inserted **b** Stapler inserted **c** Completion

Fig. 3 Method for a Billroth I anastomosis using a circular stapler during laparoscopically assisted distal gastrectomy. **a** An anvil is inserted through the incision made in the anterior wall of the duodenum. A suture placed at the posterior wall of the pylorus ring prevents the misloading of the anvil to the lumen of the stomach.

the stomach. Billroth I-type anastomosis was then performed with the circular stapler through the same incision (*Fig. 3b*). An additional Endo-GIA stapler was applied, and the resected stomach removed with lymph nodes (*Fig. 3c*).

Technique for total gastrectomy

After dissection of the gastric and gastroepiploic vessels as for the laparoscopic distal gastrectomy, the phrenoesophageal membrane and vagal nerves were divided. A further retraction tube was introduced through a 2-mm skin incision in the right hypochondrium and the abdominal oesophagus was retracted to the right side using the retraction tube and moving the patient to a right semilateral position. The short gastric vessels and posterior gastric artery were divided after ligation. The jejunum was marked 15 cm from the ligament of Treitz, and passed through the mesocolon for reconstruction. A small laparotomy was made in the upper abdomen after adequate mobilization of the oesophagus. The anvil of a 25-mm circular stapler was inserted into the distal oesophagus and the anvil of a 28-mm circular stapler was inserted into the first portion of duodenum. To fix the anvil to the abdominal oesophagus, a newly developed purse-string suture device was employed (*Fig. 4*), which was small enough to apply to the oesophagus through the small abdominal incision. The anvil was inserted into the oesophagus through an incision made in the anterior wall of the oesophagus and fixed by a thread that was placed using the miniature purse-string suture device. After the total stomach had been removed, reconstruction was performed by jejunal interposition. The 20 cm of jejunum for interposition was isolated and end-to-end jejunoduodenostomy was performed followed by an end-to-side oesophagojejunostomy using the 25-mm circular stapler. The proximal stump of the interposed intestine, through which the stapler had been inserted, was closed with an Endo-GIA stapler (*Fig. 5*).

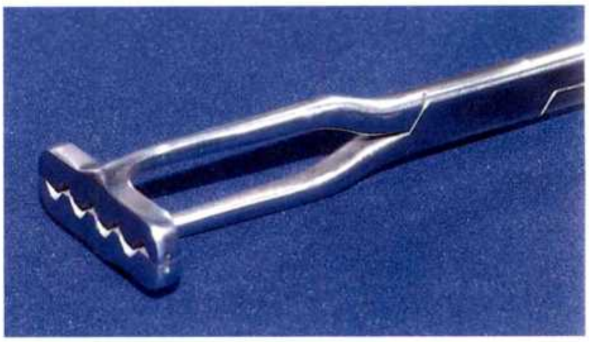


Fig. 4 Miniature (30 mm) purse-string suture device (Takasago Medical, Tokyo, Japan) for fixation of an anvil to the oesophagus

Additional lymph node dissection

When submucosal invasion was suspected on macroscopic examination of the resected specimen, the lymph nodes along with the common hepatic artery, coeliac artery and proximal side of the splenic artery[10] were dissected through the small wound after anastomosis. A C-shaped sponge was placed under the wound to prevent the liver or intestine from blocking the operative view. A ligation forceps with the same mechanism for carrying a ligature beyond vessels and a knot-pusher were employed for haemostasis.

Results

Forty-nine patients, 33 men and 16 women of mean age 60·9 (range 37–80) years, underwent laparoscopic gastrectomy. The mean operating time and blood loss were 280 (range

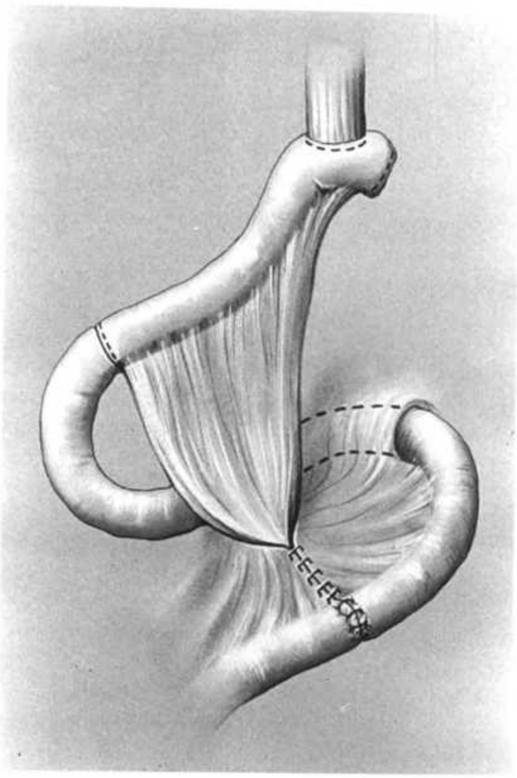


Fig. 5 Reconstruction after laparoscopically assisted total gastrectomy

Table 1 Differences between pathological and macroscopic diagnosis of depth of invasion

Pathological diagnosis of depth of invasion	*n*	Macroscopic diagnosis		
		Mucosal	Submucosa	Muscular layer
Mucosa	30 (2)	27 (2)	3	0
Submucosa	17 (3)	4 (1)	12 (2)	1
Muscular layer	2	0	1	1
Total	49 (5)	31 (3)	16 (2)	2

Values in parentheses are the number with nodal metastasis

190–510) min and 297 (range 38–1300) ml respectively. There were no cases that required conversion to laparotomy. There were no deaths. Two patients developed postoperative wound infection. One patient developed intestinal obstruction relieved by laparoscopic adhesiotomy.

The accuracy of diagnosing the depth of invasion on macroscopic examination of the specimen is shown in *Table 1*. Two patients were subsequently found to have muscular invasion on histopathological examination. Five patients, four with cancer invading the submucosa and one with cancer invading muscle, were understaged by macroscopic examination. Of 31 patients with macroscopic estimation of mucosal cancer, three had nodal metastasis.

The pathological diagnosis and status of the lymph nodes in cases with nodal metastasis is shown in *Table 2*. Five patients had lymph node metastasis, two of whom had cancer limited to the mucosa. In one patient computed tomography indicated enlargement of the lymph nodes around the common hepatic artery within 6 months of laparoscopic operation, and subsequent histopathological examination of lymph nodes resected at laparotomy confirmed metastatic disease.

Discussion

Gastric cancer is often detected early in Japan because of mass screening and endoscopic examination. Recently, the proportion of cancers invading the mucosa or submucosa has amounted to more than half the total cases requiring surgical or endoscopic treatment. In view of this situation, laparoscopic gastrectomy has been tried[5]. Because the stomach is a large organ with a rich blood flow fed by major vessels, a secure procedure requires dissection of the vessels. The present study reports the use of a ligation device and a method of gastric retraction that allows laparoscopic dissection of the lymph nodes along with the major vessels.

Table 2 Macroscopic diagnosis on resected specimen and site of metastatic nodes in patients with lymph node metastases

Patient no.	Age (years)	Sex	Macroscopic diagnosis		Pathological diagnosis	
			Depth of invasion	Node metastasis	Depth of invasion	Site of metastatic nodes
1	40	F	Mucosa	Negative	Mucosa	Perigastric, left gastric artery
2	41	F	Mucosa	Negative	Submucosa	Perigastric, common hepatic artery*
3	61	F	Submucosa	Negative	Submucosa	Perigastric
4	46	F	Submucosa	Positive	Submucosa	Perigastric
5	50	M	Mucosa	Negative	Mucosa	Perigastric

*Metastatic nodes along with the common hepatic artery were resected at reoperation

Moreover, an easy anastomosis and additional lymph node dissection were performed via a small abdominal incision, through which the resected stomach was removed. By use of these methods, the perigastric lymph nodes, along with the gastric, gastroepiploic, and common hepatic and coeliac arteries, were removed completely.

Regarding the appropriate level of lymph node dissection, historical analyses of 417 cases of conventional gastrectomy and standard D_2 lymph node dissection[10] showed lymph node metastases beyond the perigastric node in 1·3 per cent (two of 154) of patients with cancer invading the submucosa and in 18 per cent (17 of 96) of those with cancer invading muscle. These distant lymph nodes were limited to the area around the common hepatic and coeliac arteries, and no other site of lymph node metastasis was seen in cancer that invaded the submucosa. In conventional open surgery, dissection of sections 1 and 3–8, to include nodes along with the common hepatic artery, is recommended in cancer that invades the submucosa[11]. Preoperative endoscopic ultrasonography has been reported to have a diagnostic accuracy of 55 per cent in determining tumour depth[12]. Moreover, about one-fifth of patients with macroscopic stage 1 gastric cancer were reported to be understaged by intraoperative diagnosis[13]. The results of this study also show that five of the 49 patients were understaged with respect to the microscopic depth of invasion and that a misjudgement about the depth of invasion of the resected specimen could hinder curability. The lymph nodes around the common hepatic and coeliac arteries were located just under the wound from which the resected stomach was removed and could be dissected by the method described. Because of the difficulty of obtaining an accurate diagnosis of the depth of invasion and nodal metastasis, an additional lymph node dissection, which would include, at the minimum, dissection of the common hepatic and coeliac arteries, should be performed to avoid impairment of curability in a laparoscopically assisted gastrectomy for superficially invading gastric cancer.

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Original article

Selection of patients for laparoscopic repair of perforated peptic ulcer

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Background: Although randomized and non-randomized studies have evaluated the safety of laparoscopic repair for perforated peptic ulcer, no definite guidelines have been published on selection of patients for laparoscopic repair. This cohort study aimed to define patients who may not benefit from laparoscopic techniques.
Methods: The data from 374 consecutive patients with perforated peptic ulcer treated by simple repair were collected prospectively and analysed.
Results: From January 1992 to December 1998, 219 patients were treated by open suture repair, 109 by laparoscopic sutureless (fibrin glue) repair and 46 by laparoscopic suture repair. The overall leak rate after laparoscopic suture and sutureless repair was 6 and 16 per cent respectively. Leakage was noted to be associated with a significantly higher rate of wound infection, intra-abdominal abscess formation, prolonged ileus ($P < 0{\cdot}001$) and longer hospital stay (11 *versus* 5 days; $P < 0{\cdot}001$). Multivariate analysis demonstrated that Acute Physiology And Chronic Health Evaluation (APACHE) II score on admission predicted the likelihood of a leak after laparoscopic fibrin glue repair ($P = 0{\cdot}006$).
Conclusion: APACHE II score may be a useful index for selecting patients for laparoscopic fibrin glue repair.

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Introduction

Laparoscopic techniques for perforated peptic ulcers have evolved in the past few years, with the development of both sutured and sutureless repair. The authors' unit has pioneered the laparoscopic sutureless repair using fibrin glue and a gelatin plug[1], and shown it to be safe in a randomized trial[2]. Accumulating evidence suggests that laparoscopic surgery is feasible[3–8] and efficacious[2,9,10], with evidence of a reduction in wound pain and hospital stay. This study aimed to evaluate the influence of open and laparoscopic techniques on immediate treatment outcome in patients with a perforated peptic ulcer. The results might help in the selection of patients for appropriate laparoscopic repair.

Patients and methods

A consecutive series of 374 patients diagnosed before operation with a perforated peptic ulcer were evaluated prospectively. The data were recorded on a specially designed pro forma and entered into a computer. Results were analysed using the the Mann–Whitney U test, Kruskal–Wallis test and χ^2 test. Some of these patients have been reported in a previous randomized study[2].

Nasogastric tube insertion and intravenous fluid were employed routinely. Intravenous antibiotics consisted of cefuroxime 750 mg given at the time of induction and continued 8 hourly for the first day. Operations were performed under general anaesthesia with the patient supine. All operations were done by members of the on-call team when the patient was admitted. All surgeons carrying out laparoscopic repair had previous experience in laparoscopic surgery and had attended training courses, including surgery in animals and simulators. Patients who had a complicated ulcer that required definitive surgery were not included in this study.

Three simple repair techniques were employed in the present study: open suture (omental patch) repair, laparoscopic suture (omental patch) repair and laparoscopic fibrin glue repair. The choice depended entirely on the individual surgeon's preference and expertise. The laparoscopic sutureless repair using fibrin glue and a gelatin plug has been described previously[1]. Briefly, a piece of gelatin sponge (Spongostan; Ferrosan, Soeborg, Denmark) measuring 20 × 15 × 10 mm was rolled into a cone. This plug was then placed into the perforation site such that the base of the cone protruded on to the serosal surface. A 2-ml volume of prewarmed two-component fibrin sealant (Tisseel; Immuno, Vienna, Austria) was then injected via a double-lumen catheter around the plug to secure it. Peritoneal lavage was performed before completion of the operation. Each surgeon was free to convert a laparoscopic procedure to open suture repair or definitive ulcer surgery if necessary.

Postoperative management consisted of nasogastric suction and H_2-antagonist or proton pump inhibitor. All patients received analgesics in the form of intramuscular pethidine 1 mg/kg on demand. Each patient was reviewed at the same time each day by the same team of assessors to record the clincial progress and doses of pethidine required in the previous day. The team of assessors determined the timing of removal of the nasogastric tube and intravenous fluid requirements.

Patients treated by laparoscopic fibrin glue repair or laparoscopic suture repair received water-soluble contrast meal within 48 h after the operation. If a leak was demonstrated on the contrast study, the indications for reoperation depended on the clinical signs as well as the extent of the leak. Patients with a subclinical localized radiological leak were observed closely.

Patients transferred to a convalescent hospital for social reasons instead of going home were considered discharged when length of stay was considered.

This study was approved by the Ethical Committee on Human Experimentation of this hospital.

Results

Procedures

From January 1992 to December 1998, 313 men and 61 women were included in the study. The median Acute Physiology And Chronic Health Evaluation (APACHE) II score[11] on admission was 5. Two hundred and nineteen patients were treated by open suture repair, 109 patients received laparoscopic fibrin glue repair and the remaining 46 patients were treated by laparoscopic suture repair. The median (range) duration of operation for open suture, laparoscopic fibrin glue and laparoscopic suture repair was 55 (15–240), 60 (30–180) and 90 (35–165) min respectively ($P < 0{\cdot}001$, Kruskal–Wallis test). Laparoscopic fibrin glue repair was initially attempted in 149 patients; 40 required conversion to suture repair, 38 open and two laparoscopic. Laparoscopic suture repair was the planned procedure originally for 52 patients; conversion to open repair was necessary in eight patients. The overall conversion rates for laparoscopic fibrin glue repair and laparoscopic suture repair were 27 and 15 per cent respectively. The main reasons for conversion were a large (1 cm or more) ulcer perforation (22 patients) and failure to locate the perforation site despite the use of 0° and 30° lenses (15 patients). The observed difference in conversion rate between the two laparoscopic techniques did not reach statistical significance ($P = 0{\cdot}095$, χ^2 test). Conversion *per se* was not associated with an increase in mortality ($P = 0{\cdot}38$, χ^2 test) or morbidity ($P = 0{\cdot}24$, χ^2 test) rates.

Mortality rate

The overall mortality rate in this study was 9 per cent (32 of 374). Of these 32 patients, 26 underwent open suture repair, four had laparoscopic glue repair and two underwent laparoscopic suture repair. Open suture repair was associated with a significantly higher mortality rate ($P = 0{\cdot}024$, χ^2 test). Using the median APACHE II score on admission for risk stratification, patients were divided into a high-risk group (APACHE II score 5 or more) and a low-risk group (APACHE II score below 5). No patient died in the low-risk group. For the high-risk group, the mortality rates of open suture repair, laparoscopic glue repair and laparoscopic suture repair were 20, 12 and 12 per cent respectively. The association between open suture repair and higher mortality rate did not reach statistical significance after risk stratification ($P = 0{\cdot}46$, χ^2 test).

Hospital stay

Excluding patients who died, the median hospital stay was 6 (range 2–77) days. The median hospital stay of patients after open suture repair, laparoscopic glue repair and laparoscopic suture repair was 6, 5 and 5 days respectively ($P = 0{\cdot}02$, Kruskal–Wallis test). However, using the APACHE II score for patient stratification, no significant difference in median hospital stay could be demonstrated between the three repair techniques for low-risk patients (APACHE II score below 5; $P = 0{\cdot}43$, Kruskal–Wallis test) or for high-risk patients (APACHE II score 5 and above; $P = 0{\cdot}38$, Kruskal–Wallis test).

Analgesic requirement

Pethidine injections were given on demand. The median (range) dose of pethidine required was 200 (0–1725) mg after open suture repair, 75 (0–2700) mg after laparoscopic fibrin glue repair and 150 (0–1425) mg after laparoscopic suture repair ($P < 0{\cdot}001$, Kruskall–Wallis test).

Postoperative leak after laparoscopic repair

A water-soluble contrast study was arranged within 48 h after operation for patients who had laparoscopic repair. The overall leak rates after laparoscopic glue repair and laparoscopic suture repair were 16 and 6 per cent respectively. Patients with a radiologically localized leak and without signs of clinical deterioration were managed conservatively without the need for reoperation. The reoperation rates for clinical leaks after laparoscopic glue repair and laparoscopic suture repair were 10 and 4 per cent respectively. Both were significantly higher than that after open suture repair (2 per cent; $P = 0{\cdot}003$, χ^2 test). Although a leak *per se* did not affect the mortality rate ($P = 0{\cdot}31$, χ^2 test), leakage was associated with a significantly higher rate of wound infection ($P < 0{\cdot}001$), intra-abdominal abscess formation ($P < 0{\cdot}001$) and prolonged ileus ($P < 0{\cdot}001$). Moreover, patients who suffered from a leak had a longer median hospital stay (11 (range 3–77) days) than those without (5 (range 1–35) days) ($P < 0{\cdot}001$, Mann–Whitney *U* test).

Risk factors associated with a leak after laparoscopic repair

In view of the relatively high leak rate among patients undergoing laparoscopic repair, two risk factors (ulcer perforation size and APACHE II score on admission) were evaluated for their potential in predicting the likelihood of a leak. For patients undergoing laparoscopic fibrin glue repair, the mean ulcer perforation size in those who suffered from a leak was significantly higher than that in those without leakage (6·2 *versus* 5·1 mm; $P = 0{\cdot}03$, Mann–Whitney *U* test). The leak rate after repair of ulcers of 5 mm or less and more than 5 mm was 10 and 29 per cent respectively. However, ulcer perforation size was not associated with the leak rate in patients treated by laparoscopic suture repair ($P = 0{\cdot}18$, Mann–Whitney *U* test).

The median APACHE II score for patients who underwent laparoscopic glue repair complicated by leakage was significantly higher than that in those without a leak (7 *versus* 3; $P = 0{\cdot}001$, Mann–Whitney *U* test). After risk stratification, the leak rate after laparoscopic glue repair in low-risk patients (APACHE II score below 5) was 3 per cent. The leak rate did not differ significantly from those of the other two techniques of repair. However, a higher leak rate (26 per cent) was noted among the high-risk group (APACHE II score 5 or more) receiving laparoscopic glue repair ($P < 0{\cdot}001$, χ^2 test). For laparoscopic suture repair, the median APACHE II score between those with and without a leak was comparable ($P = 0{\cdot}09$, Mann–Whitney *U* test). Although univariate analysis suggested that both ulcer perforation size and APACHE II score could predict the risk of a leak after laparoscopic glue repair, only APACHE II score remained an independent predictor after multivariate analysis ($P = 0{\cdot}006$, logistic regression analysis).

Discussion

Perforated peptic ulcer is a condition for which a laparoscopic approach to repair has attractions. Not only are the site and pathology of the perforation identified, the procedure also allows closure of the perforation and adequate peritoneal lavage without a large upper abdominal incision. Various laparoscopic techniques for repair of a perforated peptic ulcer have evolved over the past few years. The beauty of laparoscopic fibrin glue repair lies in the fact that it is easily learned by relatively inexperienced laparoscopic surgeons who have not yet acquired laparoscopic suturing and knotting skills. The operation time was shorter than that of the laparoscopic suture technique (60 *versus* 90 min).

The present study was non-randomized and therefore the treatment outcomes of open *versus* laparoscopic techniques were stratified into different risk groups. In fact, no significant difference was demonstrated between open and laparoscopic repair in terms of mortality rate and hospital stay. Although the conversion rate associated with laparoscopic repair was relatively high, conversion did not account for any significant increase in morbidity or mortality rate. Instead, the postoperative leak rate was demonstrated to be associated with increased morbidity in the present analysis. The clinical leak rate after laparoscopic fibrin glue repair requiring reoperation was 10 per cent, suggesting that this might not be a safe method for all patients with a perforated peptic ulcer. The introduction of laparoscopic repair for perforated peptic ulcer aimed to minimize morbidity and shorten hospital stay; these can easily be offset by postoperative leakage. Large ulcer perforation size and higher APACHE II scores were found to be associated with higher leak rates after laparoscopic glue repair. Using multivariate analysis, the APACHE II score remained an independent predictor. Taking into consideration the high leakage rate (26 per cent) associated with this sutureless technique in high-risk patients (APACHE II score 5 or more), laparoscopic fibrin glue repair may not be safe for these patients.

Laparoscopic suture repair is a widely accepted treatment alternative for perforated peptic ulcer. Many non-randomized and randomized trials have described the safety of this technique. In this study, the leak rate after laparoscopic suture repair was higher than that after open suture repair, albeit not as high as that associated with laparoscopic glue repair. However, neither ulcer size nor APACHE II score was associated with the incidence of leaks. This was probably related to the small number of patients treated by this technique. Further study is required to identify potential risk factors to predict leaks after laparoscopic suture repair.

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Analysis of local recurrence and optimizing excision margins for cutaneous melanoma

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Background: Current guidelines for the treatment of melanoma favour conservatism; however there is still uncertainty regarding best practice for lesions of intermediate thickness. Local recurrence, a measure of treatment adequacy, can be used to determine optimum excision margins and give prognostic information for survival.

Methods: An analysis of the Auckland Melanoma Unit database was performed. Patients with local recurrence were identified and stratified by lesion thickness. Optimum excision margins were derived by regression analysis and evaluated against the database population. Survival and prognostic factors were studied.

Results: Eighty-four of 1155 patients (7 per cent) developed local recurrence. Median follow-up was 51 months. Margins predicted to give a local recurrence of zero were: 1 cm for lesions ≤ 1 mm thick; 1.5 cm for lesions 1–2 mm thick; and 2 cm for lesions > 2 mm thick. Applied to 1155 patients, there were significant differences in both local recurrence and mortality rates between optimally and suboptimally excised lesions, except for those > 4 mm thick. Thirty-three patients (39 per cent) with local recurrence died. Thickness, local recurrence and ulceration were of prognostic significance.

Conclusion: Development of local recurrence in melanomas ≤ 4 mm thick is due to inadequate treatment. It signifies progressive disease and a poor prognosis. Care must be taken to ensure that all such lesions are optimally excised.

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Introduction

The treatment of primary cutaneous melanoma has undergone considerable change over the past 15 years, as contemporary practice has become more conservative[1–4]. While complete excision is necessary, there should also be acceptable morbidity; conversely, overly conservative management may lead to an increase in recurrence rates.

There is general agreement that for lesions ≤ 1 mm thick, a 1-cm margin is sufficient[1,2]; for lesions 2–4 mm thick the accepted margin is 2 cm[3,4]. What remains unclear is the optimum margin for lesions between 1 and 2 mm thick, with one trial[1] advocating 1 cm and two trials recommending 2 cm[3,5]. The management of lesions > 4 mm thick has not yet been fully addressed.

Local recurrence is believed to be an indicator of biological aggressiveness and is also used clinically to assess the adequacy of surgical treatment[4,6]. Generally, low rates of local recurrence have been reported when using wide excision margins[1–11]. Whether this remains true with the current widespread practice of narrow excision margins remains to be seen. Local recurrence after excision of a thin lesion would be of particular concern.

Recently, the incidence of melanoma in Auckland, New Zealand was found to be higher than that of Queensland, Australia, an area previously associated with the highest incidence worldwide[12,13]. Establishment of the Auckland Melanoma Unit (AMU) database has allowed ongoing study of a number of relevant issues: optimal treatment for primary melanoma, concern that overly conservative treatment leads to increased local recurrence and mortality, and the effect of local recurrence on outcome and survival.

Patients and methods

A review of patients treated for primary cutaneous melanoma in the Auckland region of New Zealand was undertaken. The AMU database contained data from the New Zealand Cancer Registry (established in 1948, with registration mandatory since 1993[14]), from public hospital records and community laboratory databases over a 10-year period. The patients recorded in the database were treated by primary care and specialist physicians in both public and private practice (dermatology, plastic surgery, general surgery and oncological surgery).

A total of 1591 patients were eligible. Patients with lesions *in situ* and those without recorded Breslow thickness were excluded. Patients who developed a local recurrence were identified. As the database included patients treated before currently accepted treatment guidelines were adopted, local recurrence was defined as recurrence either in the wound or within 5 cm of the scar. Lesions were stratified by thickness into five groups for analysis: ≤ 1 mm, 1.01–2 mm, 2.01–3 mm, 3.01–4 mm and > 4 mm.

Both clinical and pathological excision margins were recorded for all patients. For the pathological margin, the nearest reported margin from the histological examination was used. Patients were stratified according to margin of excision in 5-mm intervals.

A logarithmic regression analysis enabled prediction of the minimum excision margins required to have a zero rate of local recurrence for each thickness group (the 'optimum margins'). These margins were then applied to the entire database of patients to test validity regarding the development of local recurrence.

Mortality rates were analysed in the following manner for each thickness: first, using the optimum margins, comparisons were made between patients whose primary lesions were optimally excised and those whose lesions were not; second, mortality rates were compared between those patients who had developed a local recurrence and those who had not.

Table 1 Local recurrence: patient data

	No.
Gender	
Men	41 (49)
Women	43 (51)
Site	
Head and neck	22 (26)
Trunk	19 (23)
Limbs	43 (51)
Thickness (mm)	
≤ 1·00	16 (19)
1·01–2·00	13 (16)
2·01–3·00	20 (24)
3·01–4·00	18 (21)
> 4·00	17 (20)
Ulceration	
Present	16 (19)
Absent	68 (81)

Values in parentheses are percentages ($n = 84$)

Survival times were defined as the time from diagnosis to recurrence (disease-free survival), and death or last follow-up (overall survival). Comparisons were made between the survival of those patients with and without local recurrence. Estimated survival distributions for disease-free survival and overall survival rates were calculated using the Kaplan–Meier method. The log rank test was used to determine the significance of differences between the curves.

The prognostic variables of gender, site of the primary lesion, ulceration and excision margins were evaluated using the Pearson χ^2-test and Fisher's exact test where appropriate. For these analyses, differences were taken to be significant at the $P < 0{\cdot}05$ level.

Results

There were 1591 patients with primary cutaneous melanoma in the database. Of these, 436 were excluded on the basis of either having a lesion *in situ* (321, 20 per cent) or having no documented Breslow thickness (115, 7 per cent). In total, 1155 patients were therefore eligible for study.

Eighty-four patients (7 per cent) developed a local recurrence (*Table 1*). The median (range) age was 66 (10–96) years and follow-up was 51 (2–262) months. The distribution of lesion thickness is shown in *Table 2*.

Excision margins

The excision margins were known for all patients. All lesions were completely excised. The results are shown in *Table 3*, given as percentages of the total lesions in each thickness group. There were no significant differences between the thickness groups in terms of the proportion of patients in each margin category.

Regression analysis for each thickness group produced the relationship between local recurrence and excision margins shown in *Figs 1–3*. From this the predicted minimum excision margins (the 'optimum margin') for a zero rate of local recurrence were: 1 cm for lesions ≤ 1 mm

Table 2 Stratification of patients by lesion thickness

Thickness (mm)	Patients	Patients with local recurrence
≤ 1·00	620 (54)	16 (3)
1·01–2·00	224 (19)	13 (6)
2·01–3·00	115 (10)	20 (17)
3·01–4·00	75 (7)	18 (24)
> 4·00	121 (10)	17 (14)
Total	1155 (100)	84 (7)

Values in parentheses are percentages

Table 3 Relationship between local recurrence and excision margins

		LR (%) by margin group (mm)					
Lesion thickness (mm)	LR	≤5	6–10	11–15	16–20	21–25	26–30
≤1·00	16 of 620 (2·6)	2·1	0·5	–	–	–	–
1·01–2·00	13 of 224 (5·8)	3·1	1·8	0·4	–	–	0·4
2·01–3·00	20 of 115 (17·4)	12·2	1·7	0·9	1·7	–	0·9
3·01–4·00	18 of 75 (24·0)	17·3	2·7	–	2·7	1·3	–
>4·00	17 of 121 (14·0)	6·6	4·1	0·8	0·8	0·8	0·8

Values in parentheses are percentages. LR, local recurrence

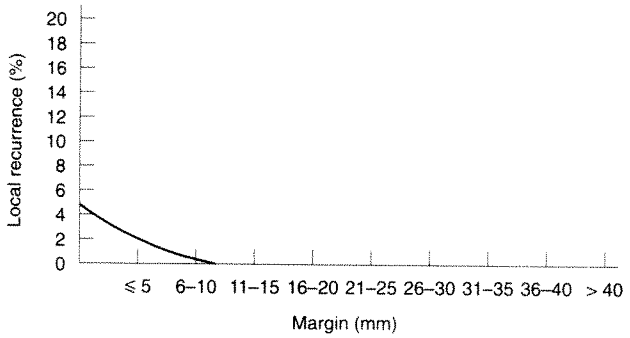

Fig. 1 The effect of excision margins on local recurrence for lesions ≤ 1 mm thick. $y = -3{\cdot}9461\ \mathrm{Ln}(x) + 4{\cdot}8352$. —, Log (≤ 1·00 mm)

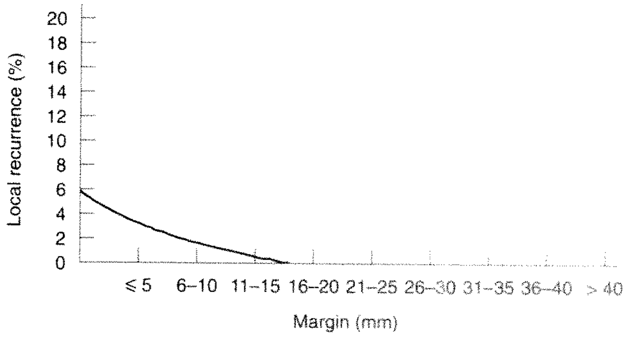

Fig. 2 The effect of excision margins on local recurrence for lesions between 1 and 2 mm thick. $y = -3{\cdot}851\ \mathrm{Ln}(x) + 5{\cdot}8452$. —, Log (1·01–2·00 mm)

thick; 1·5 cm for lesions 1·01–2 mm thick; and 2 cm for lesions > 2 mm thick.

When these predicted optimum margins were applied to the entire database of patients eligible for study ($n = 1155$), local recurrence was found to occur significantly more frequently when lesions were excised suboptimally. This was true for all lesions except those > 4 mm thick (≤ 1 mm, $P = 0{\cdot}01$; 1–2 mm and 2–3 mm, $P = 0{\cdot}04$; 3–4 mm, $P = 0{\cdot}05$; > 4 mm, $P = 0{\cdot}45$).

When the optimum margins were used to assess mortality rates, there was again a significant difference between those patients who had an optimally excised, as opposed to suboptimally excised, lesion; again this was true for all lesions except those > 4 mm thick (*Table 4*).

There were 33 deaths in the group of 84 patients with local recurrence (39 per cent). This compared with 90 deaths in the group of 1071 patients who did not develop local recurrence (8·4 per cent, $P < 0{\cdot}0001$). When the patients were stratified by lesion thickness, there was for all groups a significant difference in mortality rates between those who had local recurrence compared with those who did not, except those with lesions > 4 mm thickness (≤ 1 mm and 1–2 mm, $P < 0{\cdot}0001$; 2–3 mm, $P = 0{\cdot}05$; 3–4 mm, $P = 0{\cdot}04$; > 4 mm, $P = 0{\cdot}06$).

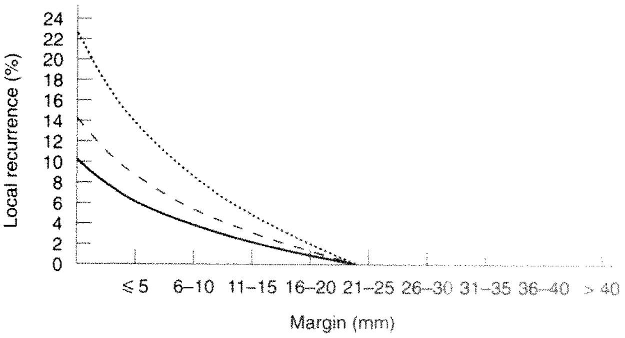

Fig. 3 The effect of excision margins on local recurrence for lesions > 2 mm thick. $y = -5{\cdot}7515\ \mathrm{Ln}(x) + 10{\cdot}188$ (2–3 mm). $y = -8{\cdot}0289\ \mathrm{Ln}(x) + 14{\cdot}292$ (3–4 mm). $y = -12{\cdot}762\ \mathrm{Ln}(x) + 22{\cdot}568$ (> 4 mm). - - -, Log (2·01–3·00 mm); ···, log (3·01–4·00 mm); —, log (> 4·00 mm)

Disease recurrence

The median (range) times to development of local recurrence were: 35 (2–262) months for patients with lesions ≤ 1 mm thick; 23 (3–118) months for lesions 1–2 mm thick; 25 (1–103) months for lesions 2–3 mm thick;

Table 4 Mortality rates in patients with optimally *versus* suboptimally excised lesions

		Optimally excised		Suboptimally excised		P*
Thickness(mm)	Deaths	LR	No LR	LR	No LR	
≤ 1·00	20	0	4	4	12	0·03
1·01–2·00	31	1	3	6	21	0·01
2·01–3·00	22	0	2	7	13	0·001
3·01–4·00	16	1	0	6	9	0·01
> 4·00	34	1	6	7	20	0·11
Total	123	3	15	30	75	

LR, local recurrence. *Fisher's exact test

24 (1–90) months for lesions 3–4 mm thick; and 10 (1–33) months for those with lesions > 4 mm thick.

Thirty-nine patients (46 per cent) manifested local recurrence alone; 12 (27 per cent) also had regional disease; five (11 per cent) also had distant disease; and seven (16 per cent) had both regional and distant disease. When stratified by thickness, 15 of 16 patients (94 per cent) in the ≤ 1 mm group had local recurrence only, and differed from the other groups with regard to more distant disease ($P < 0{\cdot}001$). For those with lesions > 1 mm thick, local recurrence was associated with more distant disease in over 50 per cent of patients, with no intergroup difference.

Survival and prognostic factors

The overall 5-year disease-free survival rate was 47 per cent, with a range of 85 per cent for patients with lesions ≤ 1 mm thick to 25 per cent for those with lesions > 4 mm thick. The 5-year overall survival rate for patients with local recurrence was 59 per cent. This compares to a 5-year overall survival rate of 86 per cent for those without local recurrence ($P < 0{\cdot}0001$).

The prognostic factors found to have a significant effect on mortality rates were local recurrence and excision margins as above, tumour thickness, and ulceration ($P = 0{\cdot}018$); age, sex and site of the primary lesion had no significant effect on survival.

Discussion

The incidence of cutaneous melanoma is increasing dramatically in New Zealand[14–16] and is a public health concern. In 1995, the crude incidence rate for the city of Auckland was 77·7/100 000[13].

Wide excision margins have traditionally been used when treating melanoma[17–21]. Recently, this practice has been questioned[7–11,22–25], and a number of randomized trials have shaped contemporary practice[1,3,5]. Veronesi *et al.*, in 1988[1] and subsequently in 1991[2], randomized patients with lesions ≤ 2 mm thick to excision margins of 1 or 3 cm, and showed no survival difference. While there was also no difference in local recurrence rates, there was a trend to suggest higher rates with narrow excision margins. Balch *et al.*[3] randomized patients differently. Patients with lesions between 1 and 4 mm thick underwent an excision with either a 2-cm or 4-cm margin. There was no survival difference. Local recurrence rates were 2·1 and 2·6 per cent, respectively[4]. Ringborg *et al.*[5] randomized patients so that patients with melanomas 0·8–2 mm thick underwent excision with either a 2-cm or 5-cm margin. Local recurrence and survival rates were similar in the two groups.

In the present study, lesions were excised by a wide range of clinicians ranging from those in primary care to those in specialist practice. While lacking the strength of a randomized trial, freedom from predefined margins allowed the entire spectrum of treatment to be studied. Histological margins were used to avoid reporting bias, and specimen shrinkage owing to processing[26–29] was also taken into account.

Patients who develop local recurrence are the best natural markers of treatment efficacy, and optimum excision margins could be derived by studying their clinical behaviour. The results of the present study confirm those of randomized trials of excision of lesions ≤ 1 mm and > 2 mm thick. An optimal excision margin of 1·5 cm was demonstrated for lesions 1–2 mm thick. An optimum margin of 2 cm was demonstrated for lesions > 4 mm thick. The validity of these margins was tested by applying them to the database of 1155 eligible patients and was found to be accurate for lesions ≤ 4 mm thick.

The present study demonstrated a high overall local recurrence rate (7 per cent), and was particularly high for lesions of intermediate thickness. In contrast, most other studies have reported recurrence rates of less than 5 per cent[1–4,7–11,22–25]. Other studies have also demonstrated a relationship between local recurrence rates and increased lesion thickness[3,4,6]. When excision margins of patients in the current study were analysed, it was clear that a large number of primary lesions were insufficiently excised. The only exception was for melanomas >4 mm thick, an observation that probably reflects biological aggressiveness. This finding emphasizes the need for adequate excision. This need becomes more apparent when mortality rates are studied. When the optimum margin was applied to overall mortality rates, a significant difference was found between patients with optimally and suboptimally excised lesions. This was observed for all thicknesses except for those >4 mm.

More than half of the patients with local recurrence had associated disease, either regional or distant metastases, or both. Local recurrence as the only manifestation was predominant in patients whose lesions were ≤ 1 mm thick. Recurrence may therefore herald the development of more aggressive tumour behaviour[6,18,19,30].

Among the prognostic factors examined, thickness, excision margins, and local recurrence significantly affected survival rates. The overall 5-year survival rates of 60 and 85 per cent for those with and without local recurrence, respectively, are highly significant. Ulceration was also found to be an indicator of poor survival, and has been consistently reported by others[3,4,26,31].

In summary, development of local recurrence in patients with primary cutaneous melanomas ≤4 mm thick is because of inadequate excision. It signifies progressive disease and poor prognosis. Care is required to ensure that all such lesions are optimally excised. Local recurrence in patients with melanomas >4 mm thick is likely to be a consequence of biologically aggressive disease and prognosis would appear to be independent of excision margins.

Acknowledgements

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Original article

The intestinal flora influences adhesion formation around surgical anastomoses

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Background: Postsurgical adhesion formation is still a cause of postoperative morbidity because no satisfactory treatment or prophylaxis has yet been developed. The present study was designed to investigate the influence of the gastrointestinal flora on adhesion formation around surgical anastomoses.

Methods: Anastomoses were constructed in germfree, conventional, ex-germfree and monocontaminated rats (*Escherichia coli* X7 and *Lactobacillus acidophilus* La5), and adhesion formation was recorded.

Results: The germfree group had a significantly lower adhesion score than all other groups ($P < 0{\cdot}05$) apart from the lactobacillus group, which had a significantly lower score than the conventional group ($P < 0{\cdot}05$).

Conclusion: The bacterial flora of the gastrointestinal tract enhances adhesion formation around surgical anastomoses.

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Introduction

Adhesions are well known complications to numerous surgical procedures. The exact elucidation of the mechanisms of postsurgical adhesion formation is still lacking and to date there is no measure or treatment that uniformly and predictably prevents or controls adhesion formation[1]. The purpose of the present investigation was to study the effect of the bowel flora and two of its constituents on adhesions around surgical anastomoses.

Materials and methods

All animal procedures were approved by the appropriate ethics committee for the use of laboratory animals.

Fourteen conventional and 34 germfree rats of both sexes of the inbred AGUS strain were used. The germfree rats were allocated to the following treatments: group 1 was kept germfree throughout the experiment ($n = 14$). Groups 2 ($n = 7$) and 3 ($n = 8$) were monocontaminated by the oral placement of a suspension containing 1×10^8 bacteria/ml of *Lactobacillus acidophilus* La5 and *Escherichia coli* X7[2,3]. Group 4 ($n = 5$) was retrieved from the germfree environment and conventionalized as described elsewhere[4]. Animals were kept in isolators[5] except for the conventional and ex-germfree rats which were kept in animal rooms with standard conditions[6]. All rats received the same steam-sterilized diet R36 (Lactamin AB, Vadstena, Sweden) and water freely. Operations were performed at 10–11 weeks of age and 6–7 weeks after flora manipulations. The microbial status of the germfree and monocontaminated animals was determined weekly.

Anaesthesia was achieved by intraperitoneal injection, 2.7 ml/kg, of a mixture of equal parts of fentanyl/fluanisone (Hypnorm®; Janssen, Saunderton, UK) in one part water and midazolam (Dormicum®; Roche, Basel, Switzerland) in one part water. The abdominal hair was cut and the abdomen was opened through a midline incision. A small part of the colon and ileum was resected 5 cm proximal to the ileocaecal junction and 2·5 cm distal to the end of the caecum, respectively. End-to-end single layer anastomoses were created with eight interrupted 6/0 polypropylene sutures (Prolene®; Ethicon, Norderstedt, Germany). The abdominal wound was closed in two layers with continuous 3/0 silk sutures. Great care was undertaken to inflict a standardized lesion in each animal and to minimize handling of the bowel as well as to avoid introduction of

Table 1 Cumulative adhesion scoring scale

0	No adhesions
+1	One adhesive band from the omentum to the target organ
+1	One adhesive band from the omentum to the abdominal scar
+1	One adhesive band from the omentum to another place
+1	One adhesive band from the adnexa/epididymal fat bodies to the target organ
+1	One adhesive band from the adnexa/epididymal fat bodies to the abdominal scar
+1	One adhesive band from the adnexa/epididymal fat bodies to another place
+1	Any adhesive band other than described above (e.g. liver to scar)
+1	Target organ adherent to the abdominal wall
+1	Target organ adherent to the abdominal scar
+1	Target organ adherent to the bowel
+1	Target organ adherent to the liver or the spleen
+1	Any other organ adherent

Each observation is given one point and the points are added to make up the score. Thus two bands are scored 1 + 1 = 2 points

foreign materials into the wound. All operations were performed in the isolator.

After 7 days the animals were killed and assessed for adhesion formation. Ileal and colonic scores were given, ranging from 1 to 5 depending on how much the adhesions covered the anastomotic circumference. These scores were then added to the score obtained by using the scoring system shown in *Table 1*[4].

Statistical analysis

The non-parametric methods of Kruskal–Wallis and Mann–Whitney U were used. A difference was considered significant at $P < 0.05$.

Results

No bacteria other than the presumed species were detected during the study period. There were no wound infections.

All rats in groups 3 and 4 and in the conventional group survived. Two of the seven in group 2 were excluded, one died on the fourth day and one had a severe anastomotic leakage. Two of the 14 germfree rats died, on the third and the sixth postoperative day, respectively. One from the germfree group was excluded because of anastomotic leakage. At necropsy dilated bowels were found but there was no true obstruction. The remaining rats were evaluated statistically.

The results are presented in detail in *Fig. 1* (as this is ordered categorical data means and standard deviations have not been calculated).

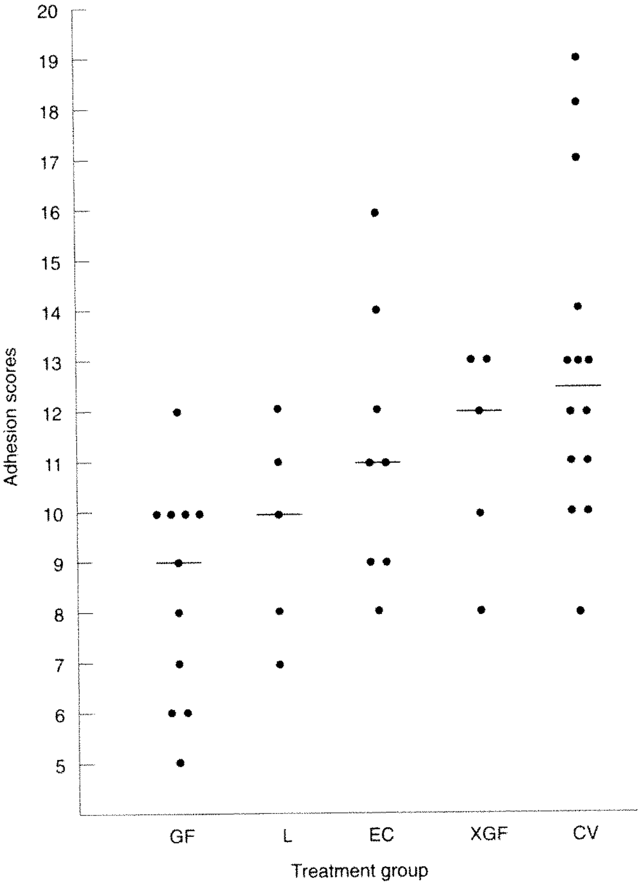

Fig. 1 Adhesion scores. Horizontal lines depict median values. GF, germfree; L, *Lactobacillus*; EC, *E. coli*; XGF, ex-germfree; CV, conventional. All groups $P = 0.0062$ (Kruskal–Wallis test). GF *versus* EC, $P = 0.0457$; GF *versus* XGF, $P = 0.0435$; GF *versus* CV, $P = 0.0007$; L *versus* CV, $P = 0.0358$ (Mann–Whitney U test)

The test groups differed significantly from each other ($P = 0.0062$). The germfree group had a significantly lower adhesion score than all other groups apart from the lactobacillus group, which had a significantly lower score than the conventional group.

Discussion

Germfree rats were compared to conventional, ex-germfree and rats monocontaminated with *E. coli* and *Lactobacillus* to determine the response to an adhesion-inducing stimulus, i.e. colonic and ileal anastomoses. The operations were all performed inside limited space steel cylinders which might have influenced the rate of complications.

It was found that the adhesion response in the germfree rats was significantly weaker compared to conventional, ex-germfree and *E. coli*-monocontaminated rats. Seemingly, restoration of the microbial environment restored the adhesion-forming ability lacking in germfree rats. The results of the monocontaminated groups indicate that *E. coli* might be more capable of restoring this effect than *Lactobacillus*. In fact, the more the flora approached the normal state the more adhesions were seen (*Fig. 1*).

No firm conclusion can be reached about how the microbes exert this adhesion-potentiating effect. Translocation of bacteria or simple leakage or migration along sutures or through small deficiencies in the anastomosis may stimulate adhesion formation.

Other possibilities might be via bacterial influence on the immune system or by modifying the tissue response to surgical trauma. It might also be through influence on healing mechanisms or on the fibrinolytic system. Interestingly, in the germfree animals there was not a complete lack of adhesion formation, indicating that factor(s) derived from the animal itself may cause adhesions, e.g. the possible ischaemic part of the experimental lesion[6]. The combination of a microbial influence and ischaemia may be the cause of adhesions frequently encountered around bowel anastomoses in clinical surgery. Moreover, the results might help to explain why recombinant tissue plasminogen activator (rtPA) works on parietal adhesions but not around anastomoses[7]. There is also a notion of two different types of adhesions, one in which fibrinolysis is crucial and that is readily prevented with, for example, rtPA, and one which occurs around colonic anastomoses and to ischaemic small intestine where rtPA has no effect[8].

The present study indicates that adhesions around anastomoses are influenced by factors from the native bowel flora as well as host factors, i.e. pertaining to the animal itself regardless of bowel flora.

Some bacteria species might be stronger promoters of the adhesion-forming process. Although antibiotics are frequently used in surgical practice it has not been established whether flora manipulations with antibiotics or probiotics could influence adhesion formation in this context. To delineate the adhesion-forming mechanism and the influence of the flora, further studies in gnotobiotic animals are warranted.

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Short note

Double near and far Prolene suture closure: a technique for abdominal wall closure after laparostomy

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Introduction

Laparostomy, leaving the peritoneal cavity open to heal by granulation, is increasingly considered to be a safe and effective technique for the management of intra-abdominal sepsis in carefully selected patients[1]. The large cavity produced by laparostomy heals by granulation from the omentum and viscera, often leaving a large abdominal defect with one or more enteric fistulas. This paper describes an experience of 17 patients with large anterior abdominal wall defects after laparostomy who underwent fistula resection and primary closure of the abdominal wall defects with interrupted double near and far Prolene (Ethicon, Edinburgh, UK) suture closure[2].

Surgical technique

Initial entry into the abdominal cavity is made, if possible, through healthy undisturbed abdominal wall above or below the defect. The neoperitoneal cavity formed after laparostomy, indicated before operation by fistula prolapse, allows sharp adhesiolysis to define the entire gastrointestinal tract including the fistula sites. Intestinal fistulas are closed by resection and standard intestinal anastomoses. A draining gastrostomy is inserted and, where possible, anastomoses are covered with surviving omentum.

After completion of the intestinal surgery, the rectus sheath is defined for 3 cm lateral to the fused edge, consisting of muscle, fascia and scar, of the laparostomy defect. A series of double interrupted no. 1 Prolene sutures is inserted with two sequences of far, near, near and far bites between the fused edges of the laparostomy defect (*Fig. 1*)[2]. A total of eight to ten double near and far sutures are inserted in each laparostomy defect and held loosely. Each suture is tied in turn with the next two adjacent sutures being held to take tension off the suture being tied. A continuous loop nylon suture is placed over the Prolene repair to approximate the edges of the defect. The skin is either loosely approximated with sutures or left open to heal by granulation.

Results

Before laparostomy the 17 patients had undergone a median of 3 (range 2–7) laparotomies for intra-abdominal sepsis. Median age was 54 (range 21–78) years, eleven of the 17 patients were men. The underlying primary disease was colorectal neoplasia in three patients, diverticular disease in three, mesenteric vascular accident in two, Crohn's disease in two patients and abdominal trauma in two. The other five patients had a variety of primary diagnoses including necrotizing pancreatitis in two, ulcerative colitis, tubo-ovarian abscess and adhesion obstruction. Reconstructive surgery was indicated in all 17 patients to close intestinal fistulas and reconstitute the abdominal wall.

The median interval between laparostomy formation and double near and far Prolene suture abdominal wall reconstruction was 8 (range 4–26) months. In 15 patients measurements from preoperative computed tomography scans were available to determine the area of the abdominal wall defect associated with the laparostomy (defect area = $3{\cdot}14ab/4$, where a is the maximum length and b the maximum width of the defect); the median area was 166 (range 55–802) cm^2. Following double near and far Prolene suture reconstruction there were no instances of postoperative death or postoperative ventilation; median hospital stay after reconstruction was 29 (range 13–90) days. After a median follow-up of 16 (range 4–61) months no patient had refistulated and only one patient has so far developed an incisional hernia.

Discussion

Previous techniques used to achieve closure of the abdominal defect associated with laparostomy include the use of lateral abdominal relaxing incisions[3] and the insertion

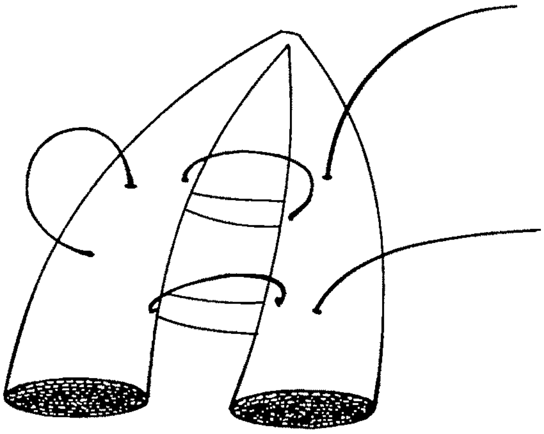

Fig. 1 Schematic diagram of an interrupted double near and far Prolene suture

of Prolene mesh. The latter risks refistulation[4] and the former technique produces further destruction of the abdominal wall which is both undesirable and can make the siting of stomas difficult.

In the experience presented here, an interrupted double near and far Prolene suture technique[2] was used for the abdominal wall reconstruction of laparostomy defects. Recently a continuous near and far suture technique after laparotomy has been associated with increased postoperative pulmonary complications and postoperative death compared with standard laparotomy closure[5]. By contrast, the reconstruction technique described here has neither been associated with postoperative ventilation nor postoperative death. In addition, after a median of 16 months' follow-up there have been no instances of refistulation and only one patient has developed an incisional hernia. The technique of double near and far Prolene suture abdominal closure should be considered in patients undergoing reconstruction of an abdominal wall laparostomy defect.

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Response of visceral peritoneum to abdominal surgery

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Background: Postoperative adhesion formation has been associated with a reduced capacity to degrade fibrin within the peritoneal cavity. Peritoneal fibrinolytic capacity has been shown to decrease during the course of a surgical operation. The aim of this study was to investigate whether tissue-type plasminogen activator (tPA), a key fibrinolytic enzyme, is released into the peritoneal cavity during operation.

Methods: Fluid released from the serosal surface of the small bowel was collected in a plastic bag from 16 patients undergoing surgery. Intraoperative blood samples were also taken from seven patients. Concentrations of the fibrinolytic components tPA and urokinase plasminogen activator (uPA), tPA activity and plasminogen activator inhibitor type 1 (PAI-1) concentration were measured by enzyme-linked immunoabsorbent assay.

Results: Intraoperative tPA concentrations were significantly raised in the peritoneal fluid collected compared with peripheral blood levels ($P = 0{\cdot}008$). This resulted in a significantly higher tPA activity in the fluid compared with blood ($P = 0{\cdot}001$). However, neither uPA ($P = 0{\cdot}29$) nor PAI-1 ($P = 0{\cdot}84$) concentrations differed significantly in fluid compared with blood.

Conclusion: These data suggest that tPA is rapidly released by the visceral peritoneum during abdominal surgery. The different concentrations in peripheral blood and peritoneum suggest that tPA is released from the peritoneum by an active process, and does not solely derive from leakage of plasma.

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Introduction

Peritoneum covers the interior of the abdominal wall (parietal peritoneum) and the visceral organs (visceral peritoneum). It consists of loose connective tissue beneath a single layer of mesothelial cells, separated by a basement membrane. Surgery damages the peritoneum, either by direct trauma to the mesothelium at the surgical site, or indirect trauma during surgical manipulation. Extravascular fibrin deposition at the peritoneal surface follows, as part of the normal inflammatory response to injury. This fibrin matrix is removed by fibrinolysis. The current model for the pathogenesis of adhesions suggests that adhesions form when fibrin is incompletely degraded, whereas when fibrinolysis is complete the serosal surface heals without adhesions[1]. Thus, disordered fibrin turnover plays a pathogenic role in peritoneal tissue repair and adhesion formation.

Experimental animal studies have supported this concept, and have demonstrated that local fibrinolytic capacity is pivotal in the early formation of adhesions[2–4]. Human mesothelial cells in culture have been shown to produce both plasminogen activators and inhibitors[5–8], with mesothelium apparently being a major source of peritoneal fibrinolytic activators[9]. Fibrinolytic capacity declines with time during laparotomy[10,11], in part because of a reduced level of tissue-type plasminogen activator (tPA), the main peritoneal fibrinolytic stimulator[12], and in part as a result of increased quenching by plasminogen activator inhibitor type 1 (PAI-1)[13]. It has been suggested, but never proven, that the reduction in tPA is due to a release of tPA into the abdominal cavity.

In order to study the peritoneal response to surgery in terms of fibrinolytic factor release into the peritoneal cavity, a method was devised to collect fluid secreted from the bowel during operation, uncontaminated by blood or irrigation solutions. The aim of the present study was to investigate whether fibrinolytic components, particularly tPA, are released into the peritoneal cavity during surgery, explaining the intraoperative peritoneal loss of tPA.

Patients and methods

Patients

Sixteen patients undergoing surgery for resection of localized colorectal cancer were included in the study. Informed consent was obtained before operation.

During the procedure, the small bowel was placed in a sterile, transparent plastic bag equipped with a purse-string closure that retained the bowel without compromising mesenteric circulation. In this manner, fluid released from the serosa of the small bowel was collected without dilution or contamination. The bag-enclosed small bowel was kept in the abdominal cavity throughout the surgical procedure, and was retracted away from the site of surgery, as would normally occur during an abdominal operation. Only patients who had an uninjured and not previously resected small bowel were included in the present study. Concomitant plasma samples were taken from seven of the patients during operation. There were ten men and six women of mean age 71 (range 45–85) years. Patients with signs of intra-abdominal infection or peritoneal carcinomatosis were not included. The volume of fluid secreted was measured.

Peripheral blood and serosal fluid samples

Venous blood was collected without stasis into three different tubes to optimize analysis of individual factors, as described previously[13]. Blood samples were immediately put on ice and centrifuged (4°C at 10 000*g*). The supernatant was aliquoted and stored at – 70°C. At the end of surgery, the small intestine was released from the plastic bag and the accumulated fluid was collected. The serosal fluid was centrifuged at 10 000*g*, and the supernatant was withdrawn, aliquoted, frozen and stored at – 70°C. Results were normalized to total protein concentration in order to allow comparison, and to avoid possible flaws due to haemodilution.

Biochemical assays

The total protein concentration was assayed in plasma and serosal fluid (DC Protein Assay; BioRad, Hercules, California, USA) according to the manufacturer's instructions. The fibrinolytic components were determined simultaneously in plasma and serosal fluid using commercially available kits. The concentrations of tPA, urokinase plasminogen activator (uPA) and the inhibitor PAI-1 were analysed by enzyme-linked immunosorbent assays (Biopool, Umeå, Sweden). The functional capacity of tPA (tPA activity) was measured using a kit from Novo Nordisk (Bagsvaerd, Denmark).

Statistical analysis

Values are given as median (interquartile range). Non-parametric tests were used: Mann–Whitney *U* test for analysing differences in plasma and peritoneal fluid from all patients included, and paired Wilcoxon signed rank test for analysing differences in plasma and peritoneal fluid concentrations in individual patients. Two-tailed $P < 0{\cdot}05$ was considered statistically significant.

Results

Clinical observations

The mean volume of serosal fluid secreted was 28 (range 3–68) ml. The small bowel was not desiccated and showed no gross signs of serosal damage such as abrasion or punctuate bleeding.

Total protein concentration

The total protein concentration in serosal fluid was lower than that in blood: 36·3 (9·8) *versus* 47·2 (8·1) mg/ml ($P = 0{\cdot}03$). However, the results obtained from statistical analysis of the normalized data did not differ from those obtained from raw data.

Serosal fluid compared with peripheral blood

The concentration of tPA was significantly raised in serosal fluid compared with peripheral blood: 1·0 (1·2) *versus* 0·1 (0·3) ng per mg protein ($P = 0{\cdot}008$) (*Fig. 1*). This resulted in significantly greater tPA activity in the serosal fluid compared with blood: 1·7 (4·5) *versus* 0·1 (0·1) fmol per mg protein ($P = 0{\cdot}001$) (*Fig. 2*). The uPA concentration was similar in serosal fluid and blood: 6·0 (7·0) and 4·0 (7·0) pg per mg protein respectively ($P = 0{\cdot}29$). There was no significant difference in PAI-1 concentration in serosal fluid and blood: 0·3 (0·1) *versus* 0·3 (0·2) ng per mg protein respectively ($P = 0{\cdot}84$).

Paired analysis

Similar results were obtained in the subset of patients from whom paired samples were collected. The concentration of tPA was significantly higher in collected serosal fluid than in peripheral blood: 0·5 (0·9) *versus* 0·1 (0·3) ng per mg protein ($P = 0{\cdot}03$). Likewise, tPA activity was significantly higher in serosal fluid: 1·4 (3·3) *versus* 0·06 (0·07) fmol per mg protein in blood ($P = 0{\cdot}02$). Intraoperative serosal fluid and blood uPA concentrations did not differ: 10·0 (30·0) and 4·0 (7·0) pg per mg protein respectively ($P = 0{\cdot}25$). PAI-1 concentrations in peritoneal fluid and blood were also similar: 0·2 (0·2) and 0·3 (0·1) ng per mg protein ($P = 0{\cdot}31$).

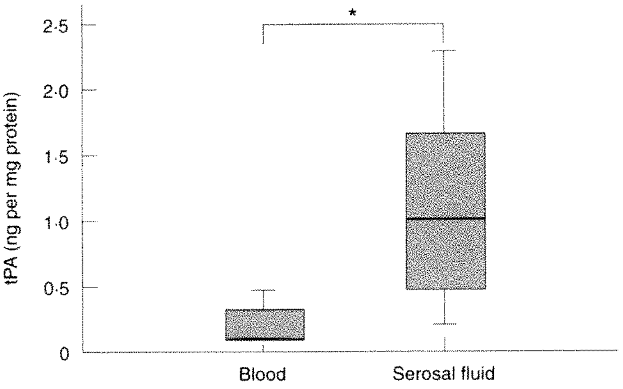

Fig. 1 Comparison between tissue-type plasminogen activator (tPA) concentration in serosal fluid and peripheral blood. Median values are represented by horizontal lines, interquartile ranges by boxes and 10–90th percentiles by error bars. *$P = 0.008$ (Mann–Whitney U test)

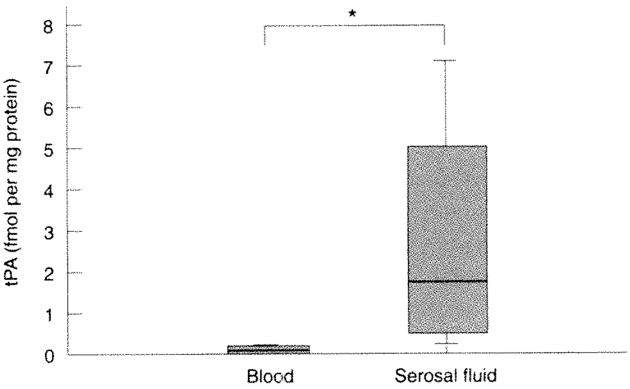

Fig. 2 Comparison between tissue-type plasminogen activator (tPA) activity in serosal fluid and peripheral blood. Median values are represented by horizontal lines, interquartile ranges by boxes and 10–90th percentiles by error bars. *$P = 0.001$ (Mann–Whitney U test)

Relative expression of plasminogen activators and inhibitors

There was no significant difference in the ratio of tPA to uPA concentration in intraoperative fluid and blood: 165·2 (246·0) *versus* 18·4 (104·3) respectively ($P = 0.06$). The ratio of tPA activity to tPA concentration was significantly higher in serosal fluid than in blood: 2·9 (2·9) *versus* 1·0 (1·4) ($P = 0.01$). Likewise, the tPA/PAI-1 ratio was significantly higher in serosal fluid than in blood (3·5 (6·2) *versus* 0·5 (1·0); $P = 0.02$) as was the ratio of tPA activity to PAI-1 (9·1 (16·3) *versus* 0·2 (0·4); $P = 0.003$).

Discussion

These results demonstrate that tPA is rapidly released by the visceral peritoneum during abdominal surgery. The fact that serosal fluid from the small bowel and blood contained different concentrations of tPA indicated that the release of tPA from visceral peritoneum occurs by an active process and is not due solely to leakage of plasma. As mesothelial cells can produce[8] and store tPA intracellularly[14], tPA can be released rapidly from the mesothelial cells into the peritoneal cavity after appropriate stimulation. Previous observations have demonstrated that the peritoneal concentration of tPA declines during surgery[10–13], and that this decline is a local response to trauma[12]. It therefore seems reasonable to assume that the release of tPA into the abdominal cavity by the small bowel serosa was a local response to the abdominal surgery.

Previous studies that have investigated peritoneal fluid by sampling drain fluid demonstrated an increased PAI-1 concentration 2–48 h after surgery[15]. The seemingly different results could be due to the different timing of sampling. An alternative explanation might be that, in the present study, fluid was collected from the serosa of the small bowel only, whereas drain fluid contains a mixture of secretions from the parietal peritoneum and visceral peritoneum, including injured and uninjured areas.

Surgery causes peritoneal injury. Tissue manipulation with accompanying mesothelial abrasion can strip mesothelial cells, which may provide the main supply of tPA intraperitoneally[9,14]. Surgery in the lower abdomen and pelvic cavity requires retraction of the small bowel, usually by covering the bowel with gauze sponges. When the gauze sponges are finally removed, there is typically visible damage to the serosa. Abrasion of the bowel with surgical gauze is frequently used in animal models (e.g. the caecal abrasion model) to create serosal damage and promote the development of adhesions[16,17]. Furthermore, it has been demonstrated in animal models that tissue protective solutions applied before such abrasive injury can significantly reduce adhesion formation[18]. It therefore seems to be of paramount importance to avoid even seemingly minor trauma to the bowel serosa. By containing the small bowel in a plastic bag, the serosa was protected from visible dehydration and damage due to manipulation.

Postoperative adhesion formation is a major source of postoperative morbidity and death[19,20]. Various devices, such as bioresorbable or permanent barriers, have been used in an attempt to prevent adhesion formation, with only limited success. There is little doubt that local fibrinolytic capacity is a decisive factor in the early development of adhesions, and that intra-abdominal administration of recombinant tPA has been used successfully in animal experiments to reduce or abolish adhesion formation[4,21]. The serosal fluid collected, which contained high levels of endogenous fibrinolytically active tPA, is typically absorbed into swabs or drained by intraoperative suction, and lost. However, if it were collected routinely, it could potentially be replaced into the abdomen at the end of surgery to counteract the postoperative fibrinolytic reduction that typically occurs[10–13], thus possibly reducing adhesion formation. The advantage of doing this would be to reduce the need for application of exogenous substances to the abdominal cavity after operation. Moreover, it may be that serosal damage leading to adhesion formation is reduced when the small bowel is contained within a plastic bag. This, however, remains to be proven.

Acknowledgements

This work was supported by grants from the Swedish Medical Research Council (project no. K98-17X-12650) and the Gothenburg Medical Society. Dr Lynn S. Peck and Dr Nasser Chegini at the University of Florida made valuable comments on the manuscript.

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Correspondence

The Editors welcome topical correspondence from readers relating to articles published in the Journal. Letters should be no more than 250 words in length and should be typed on A4-sized paper in double spacing

Hyperthermic intraperitoneal chemoperfusion in the treatment of locally advanced intra-abdominal cancer (*Br J Surg* 2000; 87: 1006-15)

Sir

We read with interest the review article concerning hyperthermic intraperitoneal chemotherapy (HIPEC) for peritoneal carcinomatosis and are pleased to see that this approach is being used in at least 30 centres worldwide. We are, however, disappointed about some of the information given. In Table 4 the authors include 385 patients, suggesting an ample experience in HIPEC treatment of colorectal carcinomatosis, but only 69 patients in the 11 studies reported are actually known to have peritoneal carcinomatosis from colorectal origin and only one of these studies (reference 96) reports separately on survival for these patients (median survival 2 years). Other not mentioned and recently updated series report a 2-year overall survival of 40-60 per cent for patients with colorectal carcinomatosis[1-4].

We have performed 135 such procedures for pseudomyxoma peritonei (53) and peritoneal dissemination from colorectal/appendiceal (71) and ovarian cancer (11). In a phase II study we treated 29 patients with colorectal cancer by HIPEC. After a median follow-up of 38 months, actuarial 1-, 2- and 3-year survival rates of 82 per cent, 45 per cent and 23 per cent were calculated. The varying natural history of this disease and the heterogeneity of the patients are such that only a randomized trial design will adequately answer the question of whether HIPEC treatment actually prolongs survival, and a randomized phase III trial is now being conducted in the Netherlands Cancer Institute.

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Authors' reply

Sir

We appreciate the interest of Dr de Bree and colleagues in our paper and we agree that the series presented in Table 4 does not consist of patients with colorectal cancer only. This simplification was made, however, with the aim of providing some structural overview of these very heterogenous studies. The recently published papers mentioned by de Bree were not available at the time of finishing our manuscript and we fully agree with the desirability of randomized trials and congratulate the authors for initiating a phase III trial on the subject.

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Role of xanthine oxidase in small bowel mucosal dysfunction after surgical stress (*Br J Surg* 2000; 87: 1094-1101)

Sir

I read with interest the article by Anup *et al.* describing the role of xanthine oxidase in small bowel mucosal dysfunction. I am concerned about the anaesthetic and analgesic regimen used in this study. Firstly, the use of ketamine (at a dose of 50 mg/kg) as the sole anaesthetic agent is recognized to be inappropriate, since although inducing immobilization of the subject, it is inadequate in providing anaesthesia for laparotomy[1]. Secondly, the authors make no mention of provision of postoperative analgesia following laparotomy. There is evidence which demonstrates that animals subjected to laparotomy alone (undertaken under appropriate inhalational anaesthesia) suffer postoperative pain which can, however, be controlled by the use of analgesics[2]. I look forward to reading the authors' reply to these observations.

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Author's reply

Sir

Thank you for the opportunity to respond to the comments raised by Dr Watkins. Ketamine has a strong analgesic effect at subanaesthetic doses, which allows it to be used as an analgesic during postoperative intensive care or as an analgesic-plus-sedative for patients receiving mechanical ventilation[1]. Intraperitoneal ketamine produces antinociceptive effects in rats[2] and ketamine anaesthesia has been used in adult male rats[3], even at a dose of 10 mg/kg body weight[4]. Ketamine produces a short-lived analgesic effect in rats even at doses of 1600 mg/kg, when administered intrathecally[5]. We have used a dose of 50 mg/kg body weight, which produced anaesthesia that was adequate for the minimal surgical intervention in our experiments.

Ketamine produces analgesia, as measured by the latency to withdraw the tail from a 55°C water bath (tail-flick latency difference)[6] and ketamine, as a dissociate anaesthetic, acts on peripheral nociceptors to produce analgesia in rats, which is related to activity of peripheral opioid receptors[7]. For our experiments, ketamine was given at 50 mg/kg body weight intraperitoneally, which would be absorbed slowly over time to provide a longer duration of anaesthesia. The animals rarely recovered from anaesthesia by 60 min after stress, at which time they were sacrificed. It is unlikely that they felt pain before sacrifice.

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Value of herniography in the management of occult hernia and chronic groin pain in adults (*Br J Surg* 2000; 87: 824-5)

Letter 1

Sir

I read with interest the comprehensive review by Fon and Spence (*Br J Surg* 2000; **87**: 545-52) and the above-mentioned paper by Calder *et al.* In the latter it is unclear how the operations were done, and whether at surgery patients operated on for a single side had the opposite groin assessed. This, I believe, can only be done laparoscopically. After experience of some 300 sportsmen undergoing a transabdominal pre-peritoneal (TAPP) repair for sportsman's hernia, it is my strong belief that sudden raised intra-abdominal pressure in the presence of an occult hernia causes an outward explosive force, tearing the external oblique aponeurosis and adjacent tissues. Rest from sport gives relief from pain, while on return to sport, symptoms recur. All patients give a typical history, but because of the lack of obvious physical signs, many suffer long delays before referral and definitive treatment. The direct stress examinations[1] confirmed the diagnosis in 96 per cent of patients.

Smedberg *et al.*[2], when examining athletes with chronic groin pain by herniography, showed an occult hernia in 49 per cent of asymptomatic groins. This figure is similar to the findings on laparoscopic TAPP repair, when the asymptomatic groin can be assessed and repaired. With follow-up extending over 8 years, and repairing asymptomatic hernias simultaneously, less than 2 per cent have returned with a further hernia problem - a very different picture to that seen after open operation.

Mesh placed at laparoscopic repair prevents excess abdominal pressure reaching the abdominal wall musculature, and allows healing. Because no muscle is cut, recovery is usually half the time following open operation, with 80 per cent of patients getting back to full sporting activities within three weeks of repair.

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Letter 2

Sir

I was interested in the short paper by Calder *et al.* One has to wonder about the cost effectiveness of a test which reveals positive results in only a quarter of the patients. I think the authors may have the same reservations in that they recommend an observation period of 4 months before proceeding to intervention. What the authors do not tell us is whether, in the patients operated on for groin pain, symptoms were relieved following repair of the occult hernia. Could we be enlightened please?

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A problem bile duct stone (*Br J Surg* 2000; 87: 838-9)

Sir

In solving the clinical dilemma of "a problem bile duct stone", Professor Van Hee offered the choice of one of three solutions; immediate conversion to open exploration, laparoscopic bile duct exploration, or postoperative endoscopic retrograde cholangiopancreatography (ERCP) to achieve common bile duct (CBD) clearance of stones detected on peroperative cholangiography. However, postoperative ERCP carries the potential risk of failure in 10 per cent of patients[1], achieves duct clearance in only 76 per cent of patients at first attempt[2], and has morbidity and mortality rates that approach 10 per cent and 1 per cent, respectively[3]. Moreover, failure of postoperative ERCP means subjecting the patient to conventional or laparoscopic CBD exploration.

An alternative solution is peroperative ERCP. This procedure is quite suitable for 'silent' stones found on routine intraoperative cholangiography. It is performed during laparoscopic cholecystectomy[4,5] and, therefore, provides a single-stage minimally invasive procedure[5]. This method is safe and duct clearance can be achieved in 85 per cent of patients[4,5]. Additional clearance can be achieved by a postoperative endoscopic procedure[5]. The only problem encountered is the extreme disorientation and difficulty encountered on attempting ERCP with the patient in a supine position but this becomes easier with experience[6]. Failure of intraoperative ERCP will leave the surgeon with the choice of laparoscopic bile duct exploration (if expertise is available) or conversion to an open laparotomy. Laparoscopic bile duct exploration, like any surgical procedure, has its own learning curve and, unfortunately, the sudden demand for ERCP CBD clearance in the laparoscopic era has meant confinement of this procedure to certain centres only.

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1 Cotton PB. Endoscopic management of bile duct stones; (apples and oranges). *Gut* 1984; **25**: 587–97.
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Clinical governance and the vascular surgeon (*Br J Surg* 2000; 87; 766-70)

Sir

I was interested to read the article by C D Irvine *et al.* I fear the difficulties of detecting underperformance by clinical governance standards may be more complex. While our usual criterion for rarity is 0·05, it is customary to perform a correction when screening a large number of variables, for example the Bonferroni correction. Would it not be reasonable to have a similar correction when looking at a number of surgeons in a district or single hospital with regard to performance (for example ten vascular surgeons)?

Without a correction, the binomial distribution tells us that, by chance, there will be a 31·5 per cent probability of at least one of those surgeons failing the test. There is a further 7·5 per cent probability of two of the surgeons failing the test by chance, and an additional 1·0 per cent probability of three surgeons failing the test. In all, an almost 40 per cent probability of at least one surgeon failing the test by chance.

Assuming there is a further surgeon who is underperforming and we set our test to have a power of 80 per cent, and the agreed rarity criteria of 0·05, then, if we apply our test to the total of 11 surgeons, again using simple probability: 12 per cent of the time (20 per cent × 60 per cent) everyone will pass the test; 48 per cent of the time the underperforming surgeons will be detected alone; 8 per cent of the time the underperforming surgeon will not be detected but one of the competent surgeons will be falsely detected; and 32 per cent of the time the underperforming surgeons will be detected but at least one of the adequately performing surgeons will also be detected. In other words only half the results will reflect the true situation (*Table 1*). This situation applies to a prevalence of approximately 10 per cent of incompetent surgeons. If the incompetent surgeons are rarer, it becomes more likely that competent surgeons will be falsely detected.

While I do not claim to have answers as to the best way forward, it is clearly most unwise to jump to conclusions when a surgeon fails a clinical governance criterion and basic statistics tell us that a correction is required when many surgeons are involved.

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Table 1 Possible outcomes of clinical governance test on 11 surgeons, one of whom is underperforming

		Underperforming surgeon†	
		Detected	Not detected
		80	20
Ten competent surgeons*			
No failures	60	48‡	12
At least one failure	40	32	8

Values are percentages. **P* value (rarity) set at 0·05; †test with power of 80 per cent to detect underperformance; ‡true situation

Authors' reply

Sir

We welcome Mr Bagley's comments. Our paper describes a statistical model that demonstrates the difficulties involved in attempting to identify under-performing surgeons using crude morbidity and mortality data. The model we use is based on statistical convention and the published literature for accepted complication rates. These parameters are open to discussion and we welcome this. We believe crude mortality rates to be inadequate for clinical governance purposes. We agree with Mr Bagley that identification of under-performing surgeons will be difficult. On the other hand, how are surgeons to be protected from inappropriate accusations or investigations into their results?

Surgeons would be less likely to be unjustly investigated by altering our criterion from 0·05 to 0·01 (equivalent to Mr Bagley's Bonferroni adjustment over five surgeons) but only at the cost of allowing poor performance to go unrecognized for longer. Alternatively strict governance could be achieved by raising the criterion from 0·05 to 0·1. The logical extension of this is that there will be more surgeons being investigated than working.

Mr Bagley's letter raises many issues. It is time to move away from using crude mortality as a stick to beat individual surgeons. Funded audit of team practice; not just mortality, be they physicians, surgeons, anaesthetists, or these groups combined, is what is required. We are unsure whether meaningful clinical governance is a cost that the National Health Service can bear.

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Bascom's procedure in the day-surgical management of symptomatic pilonidal sinus (*Br J Surg* 2000; 87: 1067–70)

Sir

We read with interest the article by Senapati *et al.* and commend the authors on their approach. We have also undertaken Bascom's procedure during the last 9 years with results that are almost identical. Of the 32 patients undergoing surgery 29 were followed-up for a median of 6 months and postoperative infection and recurrence rate were 11 and 9 per cent, respectively. In six patients the procedure was undertaken approximately 2 months following lateral incision and drainage of a pilonidal abscess in order to treat the residual midline follicles and to remove underlying hairs[1]. All operations were undertaken as day cases but under general rather than local anaesthesia. Infiltration with 30 ml of 0·25 per cent bupivacaine during operation was routine and there were no admissions or readmissions. Patients were advised to return to work within one week. All wounds were closed primarily. Although Senapati *et al.* do not close the lateral wounds, we closed all wounds primarily and did not encounter problems with this, having a similar infection rate. The operation is relatively straight forward and 10 of 32 were performed by middle-grade surgeons without a consultant present.

We concur with the authors that it is unnecessary to undertake a more radical operation for pilonidal sinus when Bascom's procedure can be undertaken as a day-case with a recurrence rate of less than 10 per cent.

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1 Bascom J. Pilonidal disease. long-term results of follicle removal. *Dis Colon Rectum* 1983; **26:** 800–7.

Analysis of 153 gunshot wounds of the liver (*Br J Surg* 2000; 87: 1030–4)

Sir

We read with interest the article by Marr *et al.* on gunshot wounds of the liver. Their data are consistent with those from the literature in that most of the injuries (70 per cent to 90 per cent) are minor (American Association for the Surgery of Trauma grades I and II[1]) which can usually be managed by simple techniques[2]. However, the authors' description of the more complex injuries affecting the remaining 45 patients (30 per cent) seems somewhat puzzling: 34 patients (23 per cent) were treated with therapeutic liver packing alone and eight patients (5 per cent) were found to have major juxtahepatic venous injuries, five undergoing primary repair.

Attempts to control bleeding at the initial operation are an understandable and natural response by surgeons[2], but recent literature suggests that manual compression of the liver wound should be the first step, followed by a more definitive assessment of the injury[2,3]. A well-established algorithm describes how a Pringle manoeuvre should follow initial manual compression[2,3]. When bleeding is arrested, the injury site may be explored; if bleeding continues, the patient should be packed and subsequent revision of the wound performed once hypothermia and coagulopathy are corrected[3]. Unfortunately, the authors do not describe outcomes of patients with higher than grade III injuries who underwent either initial packing or immediate repair.

The group suggested placing abdominal packs above and below the liver. Are packs to be placed in both the pouch of Morison and the suprahepatic area, or in both the sub- and suprahepatic area? The latter technique could compromise the blood supply to both the right kidney and gallbladder[2].

There were 238 associated intra-abdominal injuries (75 per cent) in the series, including injuries to hollow organs (duodenum 23 and colon 42) and injuries to the kidney and pancreas. They do not describe the velocity distribution of the injuries and their impact on morbidity and mortality; a high percentage of intra- or retroperitoneal bacterial (faecal) spillage probably occurred in these patients. Did the group utilize some form of temporary abdominal coverage to control visceral oedema resulting from vigorous resuscitation and massive bacterial spillage[4]?

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Authors' reply

Sir

We thank Drs Losanoff and Jones for their interest in our article. Thirty-four of 45 patients who were a subset of patients with complex gunshot liver injuries were treated using therapeutic perihepatic packing, continued resuscitation, correction of coagulation and metabolic abnormalities and subsequent pack removal. In eight patients with major venous injuries, therapeutic packing by the trauma surgeon did not control bleeding. Three of these patients had major damage to hepatic veins and retrohepatic inferior vena cava. Three other patients had complete disruption of the porta hepatis, which involved both left and right portal veins. The paper highlights the change in our liver trauma policy. The lessons learnt emphasize our recommendation in the discussion that intraoperative resuscitative packing should be used to control bleeding while an experienced hepatobiliary surgeon is summoned. The technical details of liver packing have been published previously[1]. Usually packing between diaphragm and liver is sufficient, however, after right lobe mobilization, a pack beneath the liver is useful to aid compression. We have not encountered gallbladder or renal ischaemia due to a subhepatic pack. Temporary abdominal coverage was used selectively if visceral oedema precluded initial closure or developed later.

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Book reviews

Surgery of the Breast: Principles and Art

S. L. Spear, J. W. Little, M. E. Lippman, W. C. Wood (eds)
85 × 222 mm Pp. 985. Illustrated 1998. Philadelphia. Lippincott-Raven
£197·00

To describe this volume as a mere textbook would be to underestimate its contribution. This is a comprehensive reference work for the specialist oncological, reconstructive and aesthetic breast surgeon. It comprises 72 chapters over nearly 1000 pages and is beautifully illustrated with clear pen and ink diagrams, colour diagrams and photographs. The book is divided into four useful sections covering respectively breast oncology, breast reconstruction, reduction mammaplasty and augmentation mammaplasty.

The first section covering aspects of oncology should appeal equally to the oncological surgeon and the reconstructive surgeon. The latter should be as aware of these issues as the oncological surgeon should be aware of the aesthetic issues of breast surgery that are covered in the subsequent sections. The only disappointment in this first section was the chapter on breast pathology which I felt was covered in insufficient detail either to do justice to the book as a whole or to be useful in its own right as a reference work. The chapter from the Van Nuys Group on ductal carcinoma in situ, on the other hand, was a model of clarity and concise detail that I would recommend to surgeons, non-surgical oncologists, radiologists and pathologists alike. The second section on breast reconstruction includes clear descriptions of all the major methods within the armamentarium of the reconstructive surgeon. The individual contributions sit comfortably together and illustrate that good results can be obtained in a great variety of situations by a variety of methods. The editors have been careful to avoid the pitfall of favouring more complex techniques when simple ones can have the same effect. Section three contains useful descriptions of many of the available techniques for reduction mammaplasty, including descriptions of the periareola, short vertical scar, inferior pedicle, vertical pedicle, round block and free nipple graft techniques. Some of these contributions, however, would benefit from a little more detail in the descriptions of the preoperative marking techniques which are often the key to successful execution of these procedures. Far from dismissing the topic of breast augmentation to one or two short chapters it was pleasing to see the careful detail that has been given to this topic in the final 10 chapters of the book. These cover all major aspects of this important subject, including a very good introductory chapter and a useful contribution on the psychological aspects relating to the augmentation patient.

The multiple author contributions in all these sections account for some variation in style, but the editors have done a fine job in giving the book the feel that is has been carved from a single piece rather than formed of many individual parts glued together.

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Manual of Sclerotherapy

N. S. Sadick
60 × 182 mm. Pp 272 Illustrated 2000. Philadelphia: Lippincott Williams and Wilkins £87·00.

This book is a complete manual of sclerotherapy written by Dr Sadick who is an American dermatologist/phlebologist. It is not, therefore, surprising that the book focuses on dermatological rather than surgical aspects. For a volume covering sclerotherapy, it is fairly comprehensive; chapters on anatomy, epidemiology, pathophysiology, signs and symptoms, and physical examination and noninvasive testing are included, but those chapters dealing with basic information certainly can not replace standard textbooks. The chapter on setting up a sclerotherapy suite is clearly aimed at physicians wishing to set up a sclerotherapy practice (including advertising and patient information and consent forms), rather than surgeons with a hospital-based practice. The chapter on evaluation, including physical examination, hand-held Doppler testing and photoplethysmography is detailed and well-illustrated. However, the value and accuracy of these tests could have been better commented on and they are certainly not discussed critically. Injection therapy is described and illustrated in detail. Infection is recommended for treatment of incompetent perforating veins and for reflux at the saphenofemoral junction, conditions that many surgeons would prefer to treat surgically. A short chapter 'When to ask for surgical consultation' is included but, again, many excellent surgical studies are missing from the reference list. In conclusion, this attractive-looking book is a good manual aimed at phlebologists rather than surgeons. A strong point is the many well-chosen illustrations of procedures. A weak point is the lack of up-to-date relevant references, most of which appeared before 1995 in non-surgical and non-medical journals.

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Scientific Surgery

Deriu GP, Milite D, Damiani N, Mercurio D, Bonvicini C, Lepidi S *et al.* Carotid endarterectomy without angiography: a prospective randomised pilot study. *Eur J Vasc Endovasc Surg* 2000; **20:** 250–3.

The study included 186 patients, half of whom had surgery based on the results of duplex imaging alone. The rate of death or major stroke was low, and not statistically different between the two groups 1 per cent after angiography *versus* 3·3 per cent after duplex.

Cohn SM, Lipsett PA, Buchman TG, Cheadle WG, Milsom JW, O'Marro S *et al.* Comparison of intravenous/oral ciprofloxacin plus metronidazole *versus* piperacillin/tazobactam in the treatment of complicated intra-abdominal infections. *Ann Surg* 2000; **232:** 254–62.

Some 459 patients were enrolled into this multicentre study of two different antibiotic regimens The combination of ciprofloxacin and metronidazole had a higher clinical resolution rate (74 *versus* 63 per cent) and shorter hospital stay (14 *versus* 17 days).

Hasegawa H, Yoshioka K, Keighley MRB. Randomized trial of fecal diversion for sphincter repair. *Dis Colon Rectum* 2000; **43:** 961–65.

Twenty-seven patients were studied, seven of 13 patients with a stoma had complications related to it Stoma formation did not improve the rate of wound healing or the functional outcome from the repair

Orozco H, Mercado MA, Chan C, Guillén-Navarro E, López-Martínez LZ. A comparative study of the elective treatment of variceal haemorrhage with beta-blockers, transendoscopic sclerotherapy, and surgery A prospective, controlled, and randomized trial during 10 years. *Ann Surg* 2000; **232:** 216–19.

Clinical details were similar in the three groups including a total of 119 patients. The rebleed rate was significantly reduced after surgery, particularly in patients with Child's grade A liver disease There was no significant difference in survival rates between the groups.

Luijendijk RW, Hop WCJ, van den Tol PM, deLange DCD, Braaksma MMJ, IJzermans JNM *et al.* A comparison of suture repair with mesh repair for incisional hernia. *N Engl J Med* 2000; **343:** 392–98.

Two hundred patients with a vertical midline abdominal hernia less than 6 cm in length were included Three-year cumulative recurrence rates were 43 per cent with sutures and 24 per cent after mesh repairs ($P = 0\cdot02$)

Lau JYW, Sung JJY, Lee KKC, Yung M, Wong SKH, Wu JCY *et al.* Effect of intravenous omeprazole on recurrent bleeding after endoscopic treatment of bleeding peptic ulcers *N Engl J Med* 2000; **343:** 310–16.

Some 240 patients with bleeding controlled by adrenaline injection or thermocoagulation were randomized to early high dose omeprazole infusion or placebo. All then had oral omeprazole for 8 weeks Omeprazole reduced the rate of rebleeding from 22·5 to 7 per cent and the mortality rate from 10 to 4 per cent ($P = 0\cdot13$)

Badger CMA, Peacock JL, Mortimer PS. A randomized, controlled, parallel-group clinical trial comparing multilayer bandaging followed by hosiery *versus* hosiery alone in the treatment of patients with lymphedema of the limb. *Cancer* 2000; **12:** 2832–7.

Ninety patients with unilateral arm or leg lymphoedema were enrolled Initial multilayer bandaging resulted in a significantly greater reduction in limb volume sustained at 26 weeks (31 *versus* 16 per cent, $P = 0\cdot001$)

Steinbach G, Lynch PM, Phillips RKS, Wallace MH, Hawk E, Gordon GB *et al.* The effect of celecoxib, a cyclooxygenase-2 inhibitor, in familial adenomatous polyposis. *N Engl J Med* 2000; **342:** 1946–52.

Seventy-seven patients with polyposis were enrolled. After 6 months, celecoxib 40 mg twice daily reduced the mean number of polyps by 28 per cent compared to 4 per cent by placebo ($P = 0\cdot003$).

Ballotta E, Renon L, Da Giau G, Toniato A, Baracchini C, Abbruzzese E *et al.* A prospective randomized study on bilateral carotid endarterectomy: patching *versus* eversion. *Ann Surg* 2000; **232:** 119–25.

Eighty-six patients with bilateral significant carotid disease were randomly allocated one of the two different methods for sequential operations There were no perioperative deaths and no other statistically significant differences between the methods, however, neurological event rates and restenosis were less common after eversion endarterectomy

Miller AB, To T, Baines CJ, Wall C, for the Canadian National Breast Screening Study-2: 13-year results of a randomized trial in women aged 50–59 years. *J Natl Cancer Inst* 2000; **18:** 1490–9.

This study involved nearly 40 000 women randomly allocated annual mammography with clinical examination, or examination alone. At 13 years follow-up, the mortality rate from breast cancer was identical in both groups

Douglas CD, Macpherson NE, Davidson PM, Gani JS. Randomized controlled trial of ultrasonography in diagnosis of acute appendicitis, incorporating the Alvarado score. *BMJ* 2000; **321:** 919–22.

Some 302 patients with suspected appendicitis were included. Ultrasonography reduced the time to operation, but did not affect the number of 'normal' appendixes, the perforation rate or the duration of hospital stay

Wills VL, Jorgensen JO, Hunt DR. A randomized controlled trial comparing cholecystocholangiography with cystic duct cholangiography during laparoscopic cholecystectomy. *Aust N Z J Surg* 2000; **70:** 573–7.

In the 76 patients studied, gallbladder cannulation was always successful, compared to only 72 per cent of cystic duct cannulations ($P = 0\cdot0005$) Image quality was worse and the procedure time longer after cystic duct cannulation.

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This must not exceed 200 words and should be presented in a structured format. **Background**: state why the study was done and its main aim. **Method**: describe patients, laboratory material and other methods used. Clearly identify the nature of the study, i.e. randomized controlled trial, retrospective review, experimental study, etc. **Results**: state the main findings including important numerical values. **Conclusion**: state the main conclusions but controversial or unexpected observations may be highlighted.

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Submit four copies of all illustrations and tables. Type each table on a separate page with a brief title. Line drawings are acceptable as clear black on white graphics, computer print-out or photocopies. Submit radiographs, photomicrographs, etc unmounted in the form of glossy prints, original transparencies or negatives. Illustrations in colour are encouraged and will be printed at no cost to the author. If you include photocopies, they should be of sufficient quality to enable the Journal's referees to judge their content and value. Label each illustration on the reverse side giving its number (to correspond with its reference in the text) and the name(s) of the author(s); indicate the top of the illustration. Include a measure of magnification of photomicrographs. Include explanations of symbols and shading within the figure. Survival curves must be accompanied by a table giving the actual numbers of patients involved. Include in the legends to illustrations and the footnotes to tables brief but comprehensive explanations of all the information presented. Look at recent issues of the Journal for examples of accepted layout. Any table or illustration reproduced from a published work must give the original source in full. You must obtain permission from the original author and the publisher before submission.

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Use abbreviations *sparingly*. Terms that are mentioned frequently may be abbreviated but only if this does not impair comprehension. Abbreviations must be used consistently and must be defined on first use.

Numbers and units

Use the decimal point, not a comma, for example 5·7. Use a space and not a comma after thousands and multiples thereof, for example 10 000. Use SI units (International System of Units) except for the measurement of blood pressure (mmHg).

Statistics

For detailed guidance on the handling of statistical material consult *Br J Surg* 1991; **78**: 782–4. In evaluating a manuscript the Editors and statistical referees will consider the design of the study, the presentation and analysis of data and the interpretation of results.

Design

Set out clearly the objectives of the study, identify the primary and secondary hypotheses, the chosen end-points and justify the sample size. Investigators embarking on randomized controlled studies may wish to consider the CONSORT statement (*JAMA* 1996; **276**: 637–9).

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Whenever possible use graphical presentation to illustrate the main findings of a study. The use of standard deviation and standard error should be clearly distinguished and presented in parentheses after the mean values.

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References

Type the references with double spacing in the Vancouver style (see **Preparation of manuscripts**). Reference to abstracts and personal communications is discouraged. Reference to unpublished communications will not be accepted.

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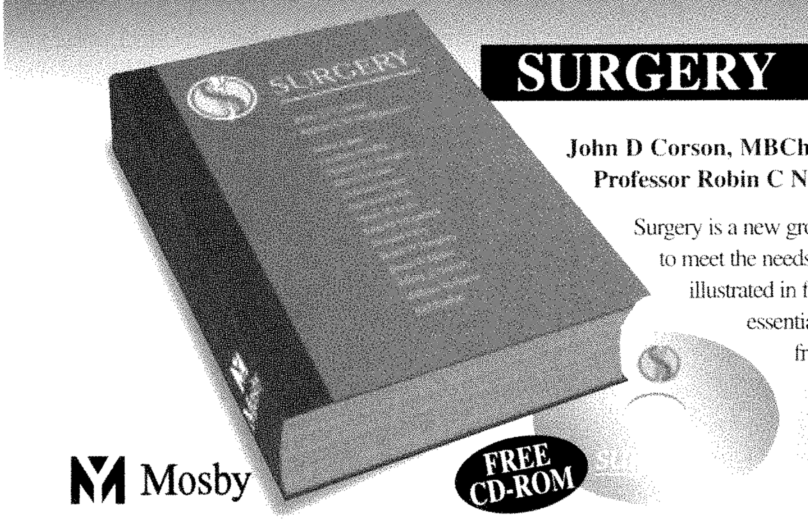

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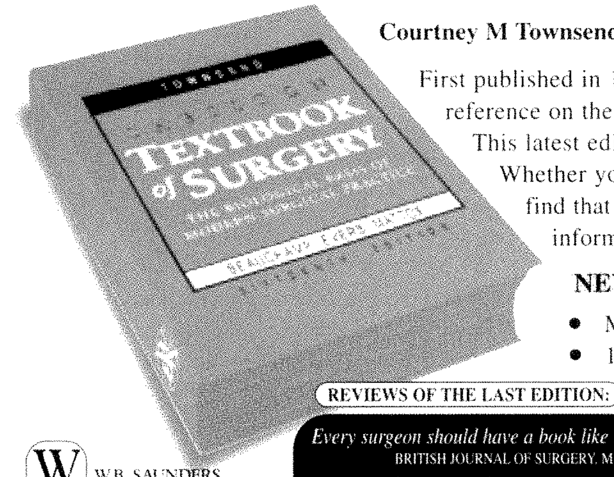

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Ethicon Endosurgery have generously funded a scholarship in memory of David Dunn. This scholarship is to the value of £3,000 and it is anticipated that this would enable a surgeon at the end of his/her training, or a consultant within 5 years of appointment, to make a substantial visit to a unit abroad to learn new skills in endoscopic surgery, with a view to introducing them to the UK. The application should include a CV, full details of the unit and the reasons for the proposed visit, together with a detailed budget of expenditure. The successful applicant will be expected to give a report on the visit at the Spring Meeting of the AESGBI.

The AESGBI are also awarding 3 travelling scholarships of £1,000. The purpose of these scholarships is to enable surgeons in training, or young consultants within 5 years of appointment, to extend their experience in minimal access surgery by short visits to one or more centres. The application should include a CV, details of the planned visit or visits, together with an estimate of the costs. One of these scholarships will be reserved for surgeons from abroad who wish to visit the UK. The successful applicants will be expected to produce a brief report of their visit.

Candidates for these scholarships should apply to the Honorary Secretary of the AESGBI (Mr David Hay) at The Royal College Surgeons of England, 35-43 Lincoln's Inn Fields, London WC2A 3PN. Candidates wishing to be considered for both types of scholarship must make separate applications for each one. The deadline for receipt of applications is 1st March 2001. The names of successful applicants will be announced at the Association of Surgeons meeting in April 2001 in Birmingham.

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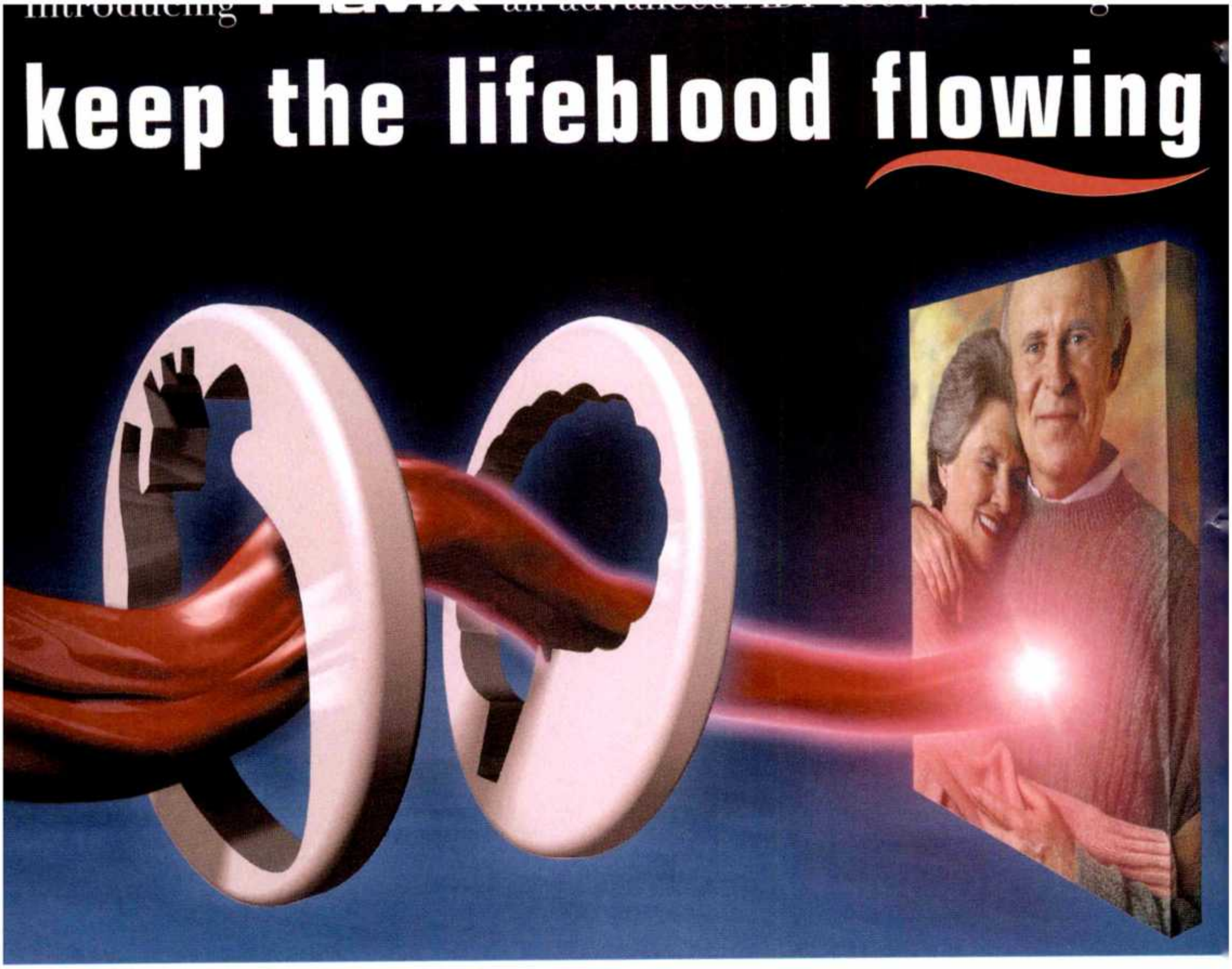

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Leading article

Probiotics

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Probiotics are living micro-organisms that on ingestion exert health benefits beyond inherent general nutrition[1]. There are increasing experimental and clinical data to support their use in the prevention and treatment of many gastrointestinal disorders, including inflammatory bowel disease, infectious and antibiotic-related diarrhoeas, and postresection disorders such as pouchitis[2,3]. In an effort to reduce the use of antibiotics in the face of increasing development of antimicrobial-resistant bacteria the World Health Organization has advocated, where possible, a policy of microbial interference therapy: the use of non-pathogens to eliminate pathogens. The ecological balance of the intestinal flora may be altered favourably by ingestion of probiotics or dietary compounds (prebiotics) that favour growth of probiotic bacteria, or both (synbiotics).

Co-evolution led to a symbiotic relationship between eukaryotes and prokaryotes with the development of sophisticated signalling systems in mucosal epithelia and lymphocytes. There are more bacteria in the intestinal lumen than there are cells in the entire human body, accounting for approximately 1 kg of intestinal content. Knowledge of the normal flora is still meagre; most of the organisms have not been characterized and at least half of the bacterial species are as yet unculturable. Metabolic activity of flora is versatile, with contributions to the synthesis of vitamins K and B complex, secondary bile acid production, fermentation of undigested residues, neutralization of dietary carcinogens such as nitrosamines, and conversion to active metabolites of certain prodrugs such as sulphasalazine. Fermentation of undigested residue in the distal small bowel and colon leads to the production of essential mucosal nutrients, such as short-chain fatty acids and certain amino acids.

The importance of the intestinal flora to intestinal development and integrity has been clearly established. Germ-free animals are delicate, have hypoplastic epithelia, impaired peristalsis and reductions in mucosal immune function and lymphoid tissue mass. Reintroduction of flora to the gnotobiotic animal restores intestinal function, mucosal proliferation, immune development and animal growth. The contribution of resident flora to mucosal defence is, therefore, not dependent simply on competition with potential pathogens but also on the exchange of regulatory and trophic signals with the host enterocytes.

Modern lifestyle changes impose stresses on systems genetically adapted over millions of years. Consumption of fermented foods has reduced dramatically and the developing mucosal immune system is exposed to a different microflora and fewer pathogens than in palaeolithic times. Modern diet and overhygienic conditions may distort development of the mucosal immune system, creating a vulnerability to intestinal infections, hypersensitivity disorders and inflammatory bowel disease[4]. Also, depending on genetic and other host-related susceptibilities, the intestinal flora may become a liability, as exemplified by intestinal overgrowth syndromes and bacterial translocation in the setting of immune or microvascular compromise.

Bacteria associated with probiotic activity are most commonly lactobacilli and bifidobacteria, but other non-pathogenic organisms, such as certain strains of *Escherichia coli* and non-bacterial organisms such as *Saccharomyces boulardii*, have been used[2]. Most of these organisms were originally obtained from faeces or gut mucosa of healthy individuals, are safe for human consumption and are available over the counter. For clinical application, probiotics must fulfil the following criteria: be of human origin, be resistant to acid and bile, be able to attach to human intestinal mucosal cells, be capable of colonization of the human intestinal tract, produce antimicrobial substances, have demonstrable probiotic activity and survive modern manufacturing processes.

Therapeutic activity is due in part to the ability of the microbe to attach to enterocytes[2,3]. This may lead to competitive displacement of intestinal pathogens, and engagement of cell membrane receptors which activate signalling events that lead to cytokine synthesis, including interferons, and cell resistance to viral attack. Probiotic organisms also alter the composition of the flora by production of lactic acid, bacteriocins and antimicrobial peptides, which are active against pathogens. Bacterial pathogens inhibited by probiotics include *E. coli*, *Streptococcus*, *Clostridium*, *Bacteroides* and *Salmonella*[2]. Additional benefits include production of mucosal micronutrients, elimination of toxins and reduction in faecal ammonia, which is toxic to the mucosa.

A role for probiotics in defence against enteric pathogens or in reconstituting the intestinal flora after antibiotic usage is conceptually sound. Probiotic lactobacilli are efficacious in treating infective diarrhoeas due to either rotavirus or *Clostridium difficile*, or associated with travel. A policy of microbial interference therapy may prevent and treat antibiotic-resistant organisms in the presence of immunosuppression or against the background of a systemic inflammatory response in the critically ill[2,3].

A rationale for treating Crohn's disease or ulcerative colitis is emerging[4,5]. A number of observations implicate some of the resident flora in driving the mucosal inflammatory response in genetically susceptible individuals. The intestinal mucosa is said to be in a state of controlled inflammation, which is finely balanced by the checking influence of regulatory T cells on effector T cell subsets. When regulation becomes defective, the activities of the effector T cells predominate and manifest as pathological inflammation. Since the normal intestinal flora varies in capacity to drive mucosal inflammation, some bacteria, such as lactobacilli and bifidobacteria without apparent pro-inflammatory activity, have been selected as candidates for probiotic treatment of inflammatory bowel disease. The results in treating different forms of murine enterocolitis are impressive, but highlight variability in probiotic performance between organisms depending on the experimental model[6,7]. Thus it cannot be assumed that a single probiotic strain will be effective in all patients or even in the same patient at different stages in the clinical course of the disease. Two controlled trials of a non-pathogenic strain of *E. coli* in ulcerative colitis have shown efficacy similar to that of mesalazine[8,9]. In acute Crohn's disease a preliminary open trial found that a lactobacillus was safe and would reduce the requirement for corticosteroids in the majority of patients[5]. Trials with well characterized strains of lactobacilli and bifidobacteria in maintenance of remission of Crohn's and ulcerative colitis are about to start in Europe.

A mixture of probiotic organisms has been used successfully in a controlled trial of maintenance treatment in patients with chronic pouchitis[10]. While combined preparations are attractive and suggest wider therapeutic advantage, more detailed information on the activity of the individual bacterial components and interstrain competition is necessary.

Probiotics may also have a role in prevention of colonic cancer and trials are now feasible for individuals who are genetically at risk[4]. Several overlapping mechanisms for their anticancer effects have emerged, including a reduction in the ability of the enteric flora to convert dietary procarcinogens to carcinogens. Other mechanisms may involve direct modulation of mucosal immune and proliferative functions. In a murine model of enterocolitis probiotic therapy was found to reduce the severity of inflammation and the rate of progression from colitis through dysplasia to invasive colonic cancer[11].

In the future further organisms will become available which will possess defined physiological functions that are applicable to maintenance of health or prevention of disease. Continued progress will require improved understanding of intestinal physiology and its relationship with the luminal ecosystem. Much of our knowledge in this area has been derived from animal models and will need to be validated in human studies. The human gastrointestinal tract differs in structure and physiology from that of other animals, and variations in lifestyle, diet and genetic constitution introduce differences in flora between individuals. Detecting and understanding these subtleties will lead to precise therapeutic targeting. At present, probiotic bacteria are safe for human consumption and seem to be effective adjuncts in the treatment of infectious or inflammatory diseases of the intestine.

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Clinical dilemma

Rectal adenocarcinoma with liver metastases: management of the primary tumour

A patient with minimal colorectal symptoms has a rectal adenocarcinoma and liver metastases. What approach should be taken to the management of the primary tumour? Mr A. Sarela and Mr D. S. O'Riordain of St James's University Hospital, Leeds, UK, reply.

The management of the minimally symptomatic primary tumour in a patient with stage IV colorectal cancer is controversial. At presentation about 25 per cent of patients with colorectal cancer have synchronous distant metastases, a substantial proportion of whom have minimal or no symptoms relating to the primary tumour[1]. Given the presence of distant disease, decisions about the management of the primary tumour are complex and require careful consideration. Fundamental to the decision-making process is accurate disease staging; indeed, the scenario of a minimally symptomatic primary tumour in a patient with stage IV cancer is the classical situation that underlines the need for routine staging before elective resection of colorectal cancer. Staging will involve, at a minimum, dual-contrast computed tomography (CT) of the abdomen and pelvis, and chest radiography. Liver lesions demonstrated by CT may require further characterization by magnetic resonance imaging (MRI), and lung nodules seen on chest radiography may require further imaging or biopsy. Performance status needs evaluation and must be considered when decisions on surgery, chemotherapy or radiation are made.

The critical decision is whether the patient is a potential candidate for curative resection of hepatic metastases. While many factors have a bearing on the oncological outcome following hepatic resection, the two principal issues in determining suitability are: (1) the potential for safe resection of all gross hepatic disease with negative margins and (2) the absence of irresectable extrahepatic disease. Up to a third of patients with liver metastases have hepatic disease only, which is potentially resectable, and such a patient should be evaluated in a specialist centre. In the best centres, major hepatic resection for colorectal metastases carries an operative mortality rate of less than 5 per cent and a 5-year survival rate of up to 40 per cent. If the patient is a candidate for hepatic resection, the primary tumour should be staged and treated in a manner identical to that in a patient without metastatic disease; this minimizes the risk of locoregional disease recurrence. Examination under anaesthesia, MRI or transanal ultrasonography will provide additional information on the tumour stage and nodal status. Neoadjuvant radiation and chemotherapy should be considered, and formal locoregional resection of the primary tumour should be performed with total mesorectal excision. An initial staging laparoscopy may be advantageous to detect undiagnosed extrahepatic disease or extensive hepatic disease, but the precise role for laparoscopy in this setting has yet to be defined. While simultaneous resection of colonic primary tumours and hepatic metastases is now commonly performed, metastases in patients with rectal cancer are generally best resected at a later date.

Where technical considerations, performance status or the presence of irresectable extrahepatic disease preclude liver resection, the focus of management should be palliative. In this scenario, management of the primary tumour is controversial. Current evidence suggests that in this situation palliative chemotherapy improves quality of life and life expectancy, and should be administered early following diagnosis[2]. Management of the primary tumour should focus on symptom control to optimize quality of life. Traditional surgical teaching advocates resection of the primary, even if minimally symptomatic, in order to preempt the future risk of intestinal obstruction, perforation or haemorrhage. However, prophylactic resection provides no immediate palliative benefit and is associated with high mortality (15 per cent) and morbidity (30 per cent) rates in patients with metastatic disease. Furthermore, surgery delays the initiation of palliative chemotherapy, reducing its beneficial effects. On the other hand, it is increasingly recognized that the majority of patients with stage IV disease, irrespective of the metastatic burden, die from systemic disease before the development of a major complication related to the primary tumour. Colonic obstruction appears to be the only significant complication of the primary tumour, occurring in 10–15 per cent of conservatively treated patients before they die[3]. Radiological assessment at initial presentation does not reliably identify which asymptomatic patients are likely to develop obstruction subsequently. A non-operative approach to the asymptomatic primary tumour with early systemic therapy in suitable patients and close clinical monitoring of the primary is the optimal approach. In the minority in whom obstruction subsequently occurs, non-surgical alternatives, such as endoluminal stenting in patients with rectal or left-sided colonic cancer, may provide good relief and avoid the need for operation.

In summary, decisions on the management of primary rectal cancer in a patient with metastatic liver disease require careful consideration based on disease staging and the potential for resection of hepatic metastases. For patients with incurable distant disease, resection of the asymptomatic primary cancer has minimal (if any) palliative benefit, can give rise to major morbidity and mortality, and inevitably delays potentially beneficial systemic therapy. Prophylactic resection should be avoided in such patients and palliative surgery should be reserved for the small proportion who subsequently develop obstruction, and then only when non-operative strategies are ineffective.

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Portal vein embolization: rationale, technique and future prospects

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Background: Advances in surgery have reduced the mortality rate after major liver resection, but complications resulting from inadequate postresection hepatic size and function remain. Portal vein embolization (PVE) was proposed to induce hypertrophy of the anticipated liver remnant in order to reduce such complications. The techniques, measurement methods and indications for this treatment remain controversial.

Methods: A Medline search was performed to identify papers reporting the use of PVE before hepatic resection. Techniques, complications and results are reviewed.

Results: Complications of PVE typically occur in less than 5 per cent of patients. No specific substance (cyanoacrylate, thrombin, coils or absolute alcohol) emerged as superior. The increase in remnant liver volume averages 12 per cent of the total liver. The morbidity rate of resection after treatment is less than 15 per cent and the mortality rate is 6–7 per cent with cirrhosis and 0–6·5 per cent without cirrhosis. Embolization is currently used for patients with a normal liver when the anticipated liver remnant volume is 25 per cent or less of the total liver volume, and for patients with compromised liver function when the liver remnant volume is 40 per cent or less.

Conclusion: This treatment does not increase the risks associated with major liver resection. It may be indicated in selected patients before major resection. Future prospective studies are needed to define more clearly the indications for this evolving technique.

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Introduction

Currently, major technical complications and fatal liver failure after hepatic resection are rare. However, complications associated with cholestasis, fluid retention and impaired synthetic function still contribute to extended hospital stay and protracted recovery following major resection[1–10]. To overcome these non-technical factors, portal vein embolization (PVE) was proposed to initiate hypertrophy of the future liver remnant before resection. This concept emerged upon recognition that portal invasion by tumour leads to ipsilateral hepatic lobar atrophy and contralateral lobar hypertrophy.

In 1986 Kinoshita *et al.*[11] published the first report demonstrating the efficacy of portal vein occlusion before resection of hepatocellular carcinoma. At that time, only arterial embolization was used, but portal tumour extension remained a problem. In an effort to minimize the risk of portal tumour emboli, Kinoshita and colleagues used PVE in conjunction with arterial embolization before resection and observed hypertrophy of the unembolized liver. This led Makuuchi *et al.*[12] to utilize PVE to initiate future liver remnant hypertrophy before extended resection for hilar bile duct cancer. Patients selected for PVE were those undergoing resection of the majority of the functional liver mass (up to 70 per cent) and whose residual liver was not cirrhotic. These studies indicated that (1) PVE is safe, (2) preoperative PVE does not complicate surgical resection and (3) liver insufficiency may be less common after resection following PVE.

Experience has provided insight into the role of PVE, but the technique and embolic substances, the method of measurement of the future liver remnant and its function, and the specific indications for PVE remain controversial. This review discusses the rationale, techniques and possible indications for this treatment.

Search strategy

An online search of the Medline database was undertaken using the keywords 'portal vein embolization', 'liver resection', 'extended liver resection', 'liver regeneration' and 'volumetric measurement' in various combinations. Boolean operators AND, OR and NOT were used. No date or language restrictions were made. Manual cross-referencing was performed.

Liver regeneration

Rous and Larimore[13] made the observation in 1920 that portal vein ligation in rabbits leads to atrophy of the ipsilateral hepatic lobe and hypertrophy of contralateral lobe. In the 1950s Schalm *et al.*[14] confirmed experimentally that this effect occurs after occlusion of the bile ducts or portal vein. Indeed, portal vein ligation[15] or occlusion resulting from tumour invasion[16,17] in humans leads to ipsilateral lobe atrophy and contralateral lobe hypertrophy. These basic observations provide the foundation for the study of liver regeneration.

The mechanisms underlying the atrophy–hypertrophy complex are poorly understood. Components essential for hypertrophy include signals to initiate, sustain and arrest regeneration. The hepatocyte has the remarkable ability to dedifferentiate and expand clonally. Stimuli leading to hypertrophy include a combination of hepatic and extrahepatic factors. Extrahepatic factors are carried primarily by the portal vein and not by the hepatic artery[18].

The physiological trigger for hypertrophy is unknown. However, the most important and powerful stimulus for regeneration is hepatocyte growth factor (HGF), which has a mitogenic effect on hepatocytes[19,20]. HGF is released from hepatocytes in response to priming factors (probably proteases) after hepatocellular injury. In concert with primary mitogenic factors (HGF, epidermal growth factor and transforming growth factor(TGF)-α[19]), essential cytokine co-mitogenic signals (tumour necrosis factor-α and interleukin-6) lead to gene induction within 30 min of a stimulus such as partial hepatectomy[21]. DNA synthesis occurs at a staggering rate for 24–72 h. Hepatocytes expand clonally, leading to an increase in cell number, not just cell mass. Mitotic index, DNA synthesis, and the number and function of mitochondria increase[22]. Indeed, cell number and total hepatic DNA in residual liver correlate with volume[23] after hypertrophy. With regard to mitochondrial function, compensatory hypertrophy is similar whether PVE or hepatic resection is the stimulus[24–26]. Insulin[27] and noradrenaline[28], and specifically portal (not arterial) hormones, are co-mitogenic with HGF[19]. Slower hypertrophy occurs in diabetics than in non-diabetics, demonstrating the clinical significance of insulin in this process[29]. The role of other co-mitogenic signals, including triiodothyronine and retinoic acid, is not well defined[19]. HGF is a much more potent stimulus for regeneration after a 'priming' stimulus, such as portal occlusion[30].

Anatomically the process begins near portal triads and spreads to the pericentral areas; work at this institution has confirmed this microscopically[31]. The hepatocytes dedifferentiate into a quasi-fetal phenotype during the process and later return to a mature form. Furthermore, the disordered cellular architecture gradually returns toward the normal adult lobular and canalicular structure[32,33].

The presumed 'stop' signals are even less well understood. TGF-β is one of several candidates for signals that lead to the arrest of cellular expansion when appropriate liver mass for the patient or animal is regained[34].

Rate of regeneration

Hepatocyte regeneration begins early after resection or PVE. The DNA index increases as early as 14 h following hepatectomy and peaks in some models at 24 h[35]. Regeneration rate is not as great in cirrhotic livers as in normal livers[17], perhaps owing to the diminished capacity of the hepatocyte in the cirrhotic liver to respond to hepatotrophic factors. Regeneration rate parallels portal venous flow rate[36].

Duncan *et al.*[31] studied hepatocyte changes in a pig model that mirrored clinical PVE. Hepatocyte replication peaked at day 7 when about 14 per cent of hepatocytes were replicating. Replication (DNA synthesis) decreased towards baseline by day 12. Computed tomography (CT) confirmed hypertrophy of the pig livers. This peak at day 7 is later than that noted after 70 per cent hepatectomy[37,38], following which the peak mitotic index occurs at days 3–4. Lee *et al.*[17] compared regeneration after PVE in Wistar rats with and without chemically induced cirrhosis. In rats with normal liver, future liver remnant weight began to increase 1 day after PVE, nearly tripled after 7 days, and reached a plateau at 7–14 days. This process was delayed and of lesser magnitude in rats with cirrhosis.

Regeneration rates reported in patients are similar to those found in animal models. Non-cirrhotic liver regenerates fastest, at 12–21 cm^3 per day 2 weeks after embolization or resection[17,29,39,40], about 11 cm^3 per day at 4 weeks[29,39] and 6 cm^3 per day at 32 days[41]. For comparison, the rate of hypertrophy is slower at 2 weeks in cirrhotic livers, only 9 cm^3 per day[17]. Nagino *et al.*[29] documented a slower rate of hypertrophy in diabetics, resembling that of patients with cirrhosis[40].

Techniques and substances for portal vein embolization

As some patients will not come to resection because of undetected metastasis or inadequate hypertrophy, a technique of PVE that minimizes complications is essential. Two major techniques have emerged to access the portal vein: direct catheterization of the ileocolic vein[42,43] and the percutaneous approach[11]. The former involves an open technique in which the ileocolic vein is cannulated at laparotomy. This method can be used when interventional radiological facilities are not available for the percutaneous approach[42]. Tumour extent can be assessed at the time of PVE but general anaesthesia and laparotomy are required, with their attendant risks[12]. The percutaneous method[44,45] is an application of Lunderquist's technique for sclerosis of oesophageal varices via the coronary vein[46]. The advantages include excellent direct access to the portal branches via an ipsilateral approach. This method minimizes the risk of portal vein thrombosis and contralateral vascular injury to the future liver remnant. Side-effects are minor and transient (much less than after arterial embolization)[12,47].

Various substances used for embolization have yielded minimally different results, with different rates and degrees of hypertrophy of the unembolized segments and associated inflammation. Cyanoacrylate and ethiodized oil, Gelfoam® (Upjohn, Kalamazoo, MI, USA) and thrombin, coils and polyvinyl alcohol (PVA), microspheres and absolute alcohol have all been used (*Table 1*). De Baere *et al.*[50] recommend cyanoacrylate as the best substance, as it leads to fast reliable hypertrophy, minimizing delay to definitive resection. Cyanoacrylate ensures portal vein occlusion, which persists after 4 weeks, whereas Gelfoam® and thrombin may allow recanalization[56]. These authors reported a 90 per cent increase in volume 30 days after cyanoacrylate embolization, compared with a 53 per cent increase 43 days after thrombin and Gelfoam®[50]. Greater inflammatory reaction was noted following cyanoacrylate embolization than after Gelfoam®[52]. Peribiliary fibrosis and casting of the portal vein may increase operative technical difficulty[50].

PVA is safe, causes little periportal reaction, and generates durable portal vein occlusion when used in combination with coils[31]. PVA is easy to use, as are Gelfoam® and thrombin, but all of these substances appear to be most useful in combination with coils. PVA or permanent particulate materials provide occlusion of small 'outflow' vessels, whereas coils provide large vessel 'inflow' occlusion. This accounts for the more permanent occlusion associated with the combination of coils and PVA[31]. PVE with absolute alcohol may be particularly useful in the treatment of hepatocellular carcinoma, although greater alteration in measured liver function occurs following embolization with alcohol compared with other substances[57]. Alcohol also leads to significant periportal necrosis and fibrosis, but recanalization is rare[58] and hypertrophy may be greatest with this substance[40].

Increasing attention is paid to embolizing not only the right portal vein but also the branches to segment IV[45,59] if an extended right lobectomy is needed. Systematic embolization of segment IV branches is important for two reasons. First, all tumour-bearing liver is embolized because accelerated tumour growth has been reported after incomplete embolization[60]. Second, systematic embolization of segment IV may contribute to better hypertrophy of segments I, II and III before extended right lobectomy (*Fig. 1*). Avoiding reflux of embolizing material into contralateral lobar veins is essential when occluding segment IV veins with any substance; bilateral or main portal vein occlusion, although never reported, remains a definite risk. Nagino *et al.*[45] recommend particulate embolization of segment IV before embolization of the right system to minimize the risk of such complications.

Pathophysiology of portal vein embolization

Makuuchi *et al.*[12] outlined the rationale for PVE before extended liver resection in patients with hilar carcinoma. First, they observed that PVE minimizes the sudden increase in portal pressure at resection, which might reduce hepatocellular damage in the residual liver. Operative manipulation of the liver, combined with changes in portal pressure, may combine to create hepatic congestion and postoperative dysfunction[61,62]. Second, they hypothesized that metabolic changes are minimized after resection following PVE as a lesser relative decrease in hepatocyte cell mass may improve overall tolerance to major resection. De Baere *et al.*[50] noted a lesser increase in portal pressure after PVE in patients with normal liver than in cirrhotics, which is consistent with data indicating a change in portal pressure after resection alone. Immediately after embolization, portal blood flow to the unembolized liver measured by transcutaneous Doppler ultrasonography increases significantly, then decreases towards (without reaching) the baseline value after 11 days[36]. The resulting hypertrophy rate correlates with flow rate measurements.

Alterations in liver function test results are minor and transient following PVE in patients with normal values before embolization. Nearly half the patients have no significant change in liver function test results after PVE. If transaminase levels or prothrombin time rise, they typically peak at a level less than three times that of the baseline 1–3 days after embolization, and return to baseline by 7–10 days[12,29,40,41,50–52] regardless of the substance used for

Table 1 Portal vein embolization: substances, techniques and volume changes

Reference	Year	No. of patients	Cancer type	Embolized substance	Time from PVE to operation (days)*	Volume of remnant† Before PVE	Volume of remnant† After PVE	Volume of remnant† Increase
Makuuchi *et al.*[48]‡	1991	54	31 HCC, 12 CCA, 8 metastatic, 3 other	Gelfoam® + Urografin® + gentamicin	14–21§	32	44	12
de Baere *et al.*[41]‡	1993	10	CRC, HCC, CCA, 2 other	Gelfoam® + thrombin (3)	43	19	31	12
				Cyanoacrylate + Lipiodol®	29			
Nagino *et al.*[44]	1993	20	GB, hilar CCA, HCC	Fibrin glue + Lipiodol® or ethanol (1)		23	43	20
Kawasaki *et al.*[49]	1994	12	Hilar CCA	Gelfoam® + Urografin® + gentamicin	17	34	42	8
Nagino *et al.*[45]	1995	4	Hilar CCA	Fibrin glue + Lipiodol®	18	31	41	10
Nagino *et al.*[29]	1995	19	14 GB, 5 CCA	Fibrin glue + Lipiodol®	14	36	47	11
de Baere *et al.*[50]	1996	31	22 CRC,1 HCC, 1 CCA, 5 other	3 Gelfoam® + thrombin	32	19	32	13
				24 Gelfoam® + thrombin + spheres		22	36	14
				4 coils		19	26	7
Shimamura *et al.*[40]	1997	7	HCC	99·5% ethanol	47	32	59	27
Wakabayashi *et al.*[51]	1997	13	HCC	Gelfoam® + coils	14	26	37	11
Imamura *et al.*[52]‡	1999	84	51 hilar CCA, 22 GB, 7 metastases, 5 HCC, 1 benign	Gelfoam® + thrombin + Urografin®	17	32	42	10
Elias *et al.*[53]‡	1999	49	27 CRC, 4 HCC, 10 other	Cyanoacrylate	32	23	35	12
Vauthey *et al.*[54]	2000	12	6 CRC, 2 CCA, 1 GB, 3 HCC	Gelfoam® + coils	35	26	35	9
Azoulay *et al.*[55]	2000	30	30 CRC	Histoacryl + Lipiodol®	63	26	37	11
Weighted average								12

*Mean interval. †Volume of functional liver remnant as a percentage of total liver volume. ‡There is some overlap of patients among these series. §Range. HCC, hepatocellular carcinoma; CRC, colorectal carcinoma; GB, gallbladder carcinoma; CCA, cholangiocarcinoma; PVE portal vein embolization; spheres, microspheres. Urografin®, Schering, Berlin, Germany. Lipiodol®, Guerbet, Aulnay-sous-Bois, France embolization. Minimal changes in total bilirubin concentration and white blood cell count are encountered.

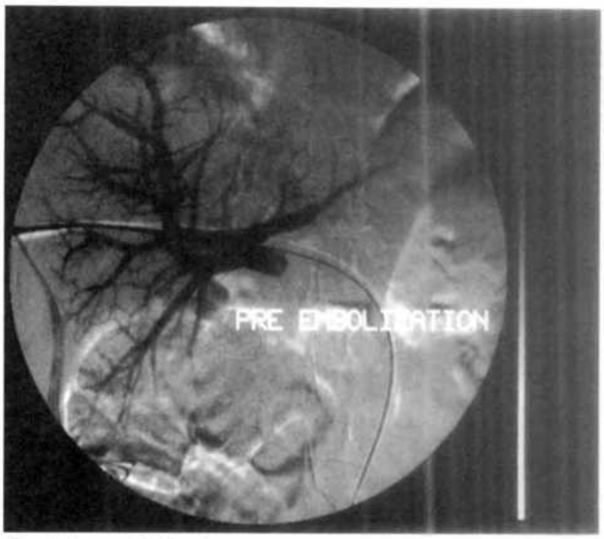
PVE is associated with minimal side-effects. Symptoms of postembolization syndrome, such as nausea and vomiting, are rare; fever and pain are minimal. This is because PVE produces no distortion of anatomy, minimal inflammation except immediately around the embolized vein, and minimal if any parenchymal or tumour necrosis[12,47]. Animal studies indicate that hepatocytes undergo apoptosis, rather than necrosis, following portal occlusion[31,63].

Volumetric measurement

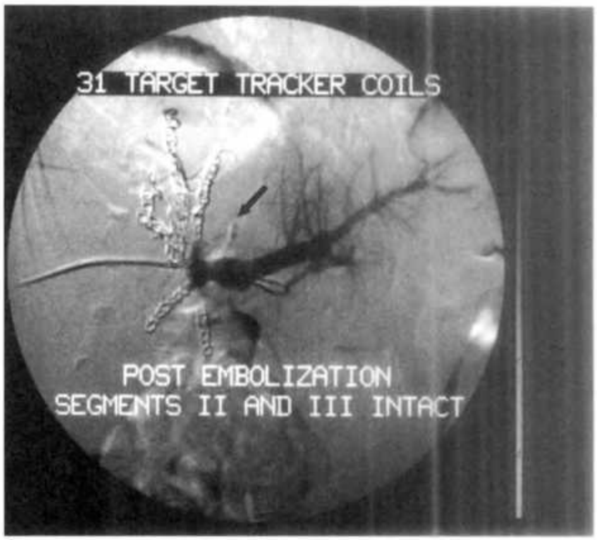
CT is the cornerstone of surgical planning; it can reproducibly[64] and accurately[65–67] measure liver volume. Ultrasonographic volumetric measurement has not been found to be accurate[64,67]. The standard technique measures resected and total liver volumes, and estimates the 'normal liver' by deducting tumour volume: (resected volume – tumour volume)/(total liver volume – tumour volume)[68,69]. With multiple tumours the technique is demanding and the calculations become imprecise because of the cumulative error rate associated with multiple measurements. In addition, the method does not account for the actual functional liver volume when the bile ducts are dilated or the liver parenchyma is compromised by vascular obstruction[54,68,69].

An alternative method of assessing the effect of PVE measures the future liver remnant directly with CT and estimates the total liver volume by means of a formula[54]. The ratio of future remnant : total estimated liver is calculated. The formula is derived from the close association between total liver volume and body surface area (BSA) as described previously[70] (total liver volume = $706 \cdot 2 \times \text{BSA} + 2 \cdot 4$). This alternative method allows for a uniform comparison of remnant volume before extended resection, with or without preoperative PVE. Based on this method of calculation, a correlation between future liver remnant volume and operative outcome has been established[54].

Surgical decision-making can be appropriately influenced by CT volumetric analysis[65]. If the remnant is estimated to be too small, the decision to utilize PVE to increase its size can be made. This technique has been used accurately to assess segmental graft volume of donors for living related liver transplantation[71,72]. Preoperative estimated liver volume based on CT volumetric analysis correlates with resected specimen volume[67,69]. Specifically, CT accurately measures liver volume after PVE[65]. Errors in CT volume calculation include the partial volume effect, respiratory phase and interobserver variation, but refinement of technique has minimized these problems[64,67]. Given these sources of error, volumetric accuracy is within ± 5 per cent[66].



a Before embolization



b After embolization

Fig. 1 Percutaneous transhepatic portogram **a** before and **b** after right trisectoral portal vein embolization. In **b** the coils outline portal vein branches to the right lobe and segment IV (arrow)

Physiological studies to complement volumetric study can be used before and after resection to estimate hepatocyte function. Many tests have been evaluated, but none reliably predicts future hepatic function. Indocyanine green (ICG) is a dye that is cleared from the circulation by the liver. Its clearance can be used as an indicator of hepatocyte function. Retention rate 15 min after intravenous injection of ICG (0·5 mg/kg) correlates with outcome in some series[51,73,74]. ICG excretion and bile volume from the unembolized lobe increase after PVE, and

Table 2 Complications of embolization and resection following portal vein embolization

Reference	Year	No. of patients	Cancer type	Major PVE complications	Percentage of embolized patients who had resection	Major perioperative complications*	30-day operative deaths
Kinoshita *et al.*[11]	1986	21	HCC	0	100		NR
Makuuchi *et al.*[12]†	1990	14	Hilar CCA	0	100	2 abscess, 2 hepatitis	See Imamura *et al.*[52]
Makuuchi *et al.*[48]†	1991	54	31 HCC, 12 CCA, 8 metastatic, 3 other	0	85	3 abscess,1 pneumonia	1 (cholangitis); see Imamura *et al.*[52]
de Baere *et al.*[41]†	1993	10	CRC, HCC, CCA, 2 other	1 required re-embolization	90		See de Baere *et al.*[50]
Lee *et al.*[17]	1993	29	HCC	NR	83		0 of 10 with no cirrhosis 1 of 14 with cirrhosis
Nagino *et al.*[44]	1993	20	GB, hilar CCA, HCC	1 transient haemobilia	NR		NR
Kawasaki *et al.*[49]	1994	12	Hilar CCA	0	100	1 abscess, 1 pancreatic leak	0
Nagino *et al.*[45]	1995	4	Hilar CCA	0	NR	1 transient liver insufficiency	0
Nagino *et al.*[29]	1995	19	14 GB, 5 CCA	NR	68		NR
de Baere *et al.*[50]†	1996	31	22 CRC, 1 HCC, 1 CCA, 5 other	1 flushing (carcinoid)	77	1 ATN, 1 bile leak	2 portal vein thrombosis with MOF and pneumonia; see Elias *et al.*[53]
Shimamura *et al.*[40]	1997	7	HCC	0	100	1 bile leak, 1 abscess, 2 liver insufficiency	0
Wakabayashi *et al.*[51]	1997	13	HCC	0	100	5 prolonged jaundice	2 liver failure
Imamura *et al.*[52]†	1999	84	51 hilar CCA, 22 GB, 7 metastases, 5 HCC, 1 benign	2 SBO, 1 reoperation (for SBO)	79		1 bleeding after liver failure
Elias *et al.*[53]†	1999	49	27 CRC, 4 HCC, 10 other	0	84	2 bile leak, 1 abscess, 2 GI anastomotic leaks‡	1 peritonitis‡ (1 at 42 days from sepsis)
Vauthey *et al.*[54]	2000	12	6 CRC, 2 CCA, 1 GB, 3 HCC	0	58	1 ascites and wound breakdown	0
Azoulay *et al.*[55]	2000	30	30 CRC	1 (hepatic artery injury, septic liver necrosis)	63	1 technical (unrelated to PVE), 2 biliary fistulas	0 (1 death at 45 days)

*Other than death. †There is some overlap of patients among these series but complications are listed only once. ‡Complication related to intraperitoneal chemotherapy and bowel resection at the time of liver resection, not a complication of liver resection. PVE, portal vein embolization; NR, not reported; HCC, hepatocellular carcinoma; CRC, colorectal carcinoma; GB, gallbladder carcinoma; CCA, cholangiocarcinoma; MOF, multiorgan failure; GI, gastrointestinal; SBO, small bowel obstruction; ATN, acute tubular necrosis correlate with volume increase of that lobe[75]. Others suggest that radiocolloid liver scintigraphy with technetium-99m–galactosyl human serum albumin is more accurate than ICG clearance[76,77]. However, the only consistently used test is CT volume estimation. Standardizing liver size measured by CT to body surface area may be the simplest, most reproducible, method of planning[54,70]. ICG clearance may be most helpful as an adjunct to the necessary CT volumetry only when underlying liver is compromised.

Indications for portal vein embolization

General indications for PVE are based on the size of the future liver remnant, expected function of the remnant, and extent or complexity of the procedure. Some primary and secondary hepatic tumours, benign and malignant, are amenable to extended hepatic resection. In patients with portal vein invasion by tumour or portal vein thrombosis, PVE is not necessary. PVE may be most useful in patients with multiple small tumours without contralateral regeneration as a result of tumour growth.

Few data exist regarding the sufficient hepatic mass or volume required to support life and prevent complications. The general consensus that, for normal livers, 25 per cent of the total hepatic volume needs to be preserved to make resection safe[41,50] has been determined empirically. In a recent series, remnant volume was calculated in 20 patients without underlying chronic liver disease before extended right lobectomy[54]. In 15 patients who underwent the anticipated resection, a future liver remnant of 25 per cent or less was associated with an increased length of hospital stay and an increase in the rate of postoperative complications. The function of liver compromised by cholestasis, inflammation, steatosis, fibrosis or cirrhosis is variable. A larger remnant is required to avoid posthepatectomy insufficiency or failure in patients with compromised liver function. Two recent studies suggest that 40 per cent of the total hepatic volume should remain for liver compromised by underlying chronic liver disease[68] or high-dose chemotherapy[55] in order to minimize complications.

Survival from irresectable metastatic colorectal cancer to the liver is dismal[78,79], but a 5-year survival rate of 30 per cent is achieved when metastases are resected[80]. Unfortunately, only 5–10 per cent of patients with metastatic colorectal cancer are candidates for liver resection, often as a result of multisegment involvement[81,82]. Hepatic resection for hilar bile duct cancer is advocated to achieve negative margins[5,83–85], but postoperative complications remain significant[4,5,86,87]. Only 30 per cent of patients with hepatocellular carcinoma can be treated by resection, partly because of stage at presentation, but also due to the background of cirrhosis in which these tumours most often arise[88]. In each of these instances, PVE may be indicated in selected patients before resection if a small remnant is anticipated. Over a period of 8 years Azoulay *et al.*[55] used PVE before major resection (four or more segments) in 19 patients heavily pretreated with chemotherapy for metastatic colorectal cancer. This was a highly selected group of patients as a further 88 major resections for metastatic colorectal carcinoma without PVE were performed at the same centre over the same period.

Another novel indication for PVE may be to induce hepatocyte replication to enable hepatic gene therapy. Hepatic gene therapy is being investigated for treatment of serum protein deficiencies, including haemophilia and metabolic defects. Gene therapy delivery vehicles under investigation provide only transient transgene expression in the liver. Retroviral delivery systems have the advantage of providing more durable transgene expression, but require cell replication to enable transfection. Thus, limited PVE may play a role in future hepatic gene therapy with retroviral delivery systems[31].

Complications and results of resection following portal vein embolization

Results of PVE are presented in *Table 1*. In spite of differences in disease type resected, embolization substances and embolization technique, and time interval from PVE to resection (14–63 days), results are remarkably similar. Predicted future liver remnant volume increased from 19–36 per cent of total liver volume before embolization to 31–59 per cent after embolization. Complications of PVE were few (zero to 10 per cent) and included the need for re-embolization[41], transient haemobilia[44] and small bowel obstruction[52]. No major complications were reported. Some 58–100 per cent of patients who underwent preoperative PVE eventually had a resection.

About 250 liver resections have been reported following PVE (*Table 2*). The perioperative mortality rate is 0–6·5 per cent in patients without cirrhosis[17,40,45,49,52] and 6–7 per cent in those with cirrhosis[12,17,44,50,51]. Complications occurred in less than 15 per cent. This complication rate is equal to or lower than the rate currently reported for major resection without PVE[1–10]. These data also suggest that borderline candidates for resection (based on small remnant size) may undergo resection without increased surgical risk following PVE.

Few studies have reported detailed follow-up. Wakabayashi *et al.*[51] reported 69 and 40 per cent 1- and 2-year survival rates, respectively, in a small series of resections for hepatocellular carcinoma. Elias *et al.*[53] reported an overall 5-year survival rate of 31 per cent (including two postoperative deaths) in 41 operated patients. A subgroup of 27 patients with colorectal metastases who would have been irresectable without PVE achieved an overall 5-year survival rate of 29 per cent. Azoulay *et al.*[55] recently reported a 40 per cent 5-year actuarial survival rate after resection following PVE, compared with a 38 per cent 5-year actuarial survival rate following resection in a cohort with similar extent of resection without PVE.

Conclusion

PVE may not only increase the pool of patients who are candidates for liver resection, but may also increase the safety of resection. Liver hypertrophy relies on the ability of the hepatocytes to dedifferentiate, expand clonally and respond to hepatotrophic stimuli, which are predominantly portal in origin. Growth factors, cytokines and hormones, especially HGF and insulin, are essential in the process.

Substances for embolization yield similar results in terms of hypertrophy, and no specific substance emerges as universally superior to the others. The authors' bias is to use a combination of coils and PVA. A period of 2–4 weeks is sufficient to enable adequate hypertrophy for normal livers, minimizing the delay to resection in patients with malignancy. Cirrhotics and diabetics require prolonged periods (6–8 weeks) to hypertrophy and may not ultimately experience the degree of hypertrophy possible in patients with normal liver.

There is a risk that PVE will be overutilized. This risk stems primarily from the present inability to predict accurately the function of the future liver remnant. Some estimate that 40 per cent of the total liver volume must be preserved to make resection safe when the liver has been compromised[55,68]. This cut-off may be higher than necessary and result in a significant number of unnecessary embolizations. Certainly, in patients with normal liver, preliminary data suggest that a remnant of more than 25 per cent of the total liver volume is acceptable, and PVE is not indicated in such patients[54]. In future studies the prediction of postoperative complications should be based on an algorithm combining volumetric measurement and grading for liver disease and function (inflammation, steatosis, fibrosis). Such studies should determine the response to PVE and indicate the minimum acceptable safe volume for different subsets of patients.

A randomized trial cannot be recommended to test the efficacy of PVE, for it would be unethical to deny the benefit of the technique and safer resection to patients who are otherwise poor candidates for resection based on inadequate liver size or function. Continued prospective examination of PVE and liver volumetric measurement, with defined endpoints such as liver function, operative complications and length of stay, should clarify the additional benefits provided by preoperative embolization.

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Review

Positron emission tomography and colorectal cancer

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Background: The oncological applications of positron emission tomography (PET) have gained widespread acceptance. This rapidly evolving technology has been applied successfully to colorectal cancer, but has not yet become part of routine clinical practice. This review considers (1) the biological basis for the use of PET in colorectal cancer, (2) the technical aspects of PET relevant to the referring clinician and (3) the application of PET to the management of primary and recurrent disease.
Methods: A Medline database search was performed for the period 1980–2000. Experience was also drawn from the first 40 patients with colorectal cancer investigated at this institution.
Results and conclusion: PET has a proven role, and is cost effective in the management of recurrent cancer and the monitoring of therapy. However, further evaluation is still required to justify its routine use for other indications in colorectal cancer. Development of new positron-labelled radiopharmaceuticals, in parallel with advances in detector technology and innovative models for tracer production and distribution, means that the availability of PET and its applications in the management of colorectal cancer will expand over the coming years.

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Introduction

Colorectal cancer is the second commonest cancer to occur both in the European Community[1] and in the USA[2,3]. The disease kills one in every two people affected. Despite the continuing advances in therapeutic strategies, both surgical and non-surgical, recurrence rates after initial treatment are estimated to be 30–40 per cent; recurrence usually occurs within the first 2 years of treatment[3–5]. There is weakness in the initial staging process and there is evidence that current methods of imaging patients with colorectal cancer are not sufficiently accurate[6]. Computed tomography (CT) forms part of the staging process for primary cancer, but it has a low staging sensitivity[7]. Intraoperative ultrasonography improves the detection of liver lesions, but this information cannot be provided before operation and so cannot contribute to preoperative patient management[8].

Detection of recurrence can also be difficult. Serial measurement of carcinoembryonic antigen (CEA) concentration in the blood is the most frequently used method for detection of asymptomatic recurrence[9], but both sensitivity (59 per cent) and specificity (84 per cent) are low[10]. The routine use of CT for postoperative surveillance is conventional, but it has not been shown to be either sensitive or specific[6,11,12]. Although magnetic resonance imaging (MRI) may augment CT in differentiating local recurrence from scar tissue in the pelvis[13,14], limitations still exist in terms of specificity and size of tumour detected[15]. Additionally, the dilemma remains of what course to take when the CEA level rises in association with minimal symptoms and normal imaging.

Positron emission tomography (PET) is beginning to make an impact in the UK. This technology couples picomolar sensitivity with high-resolution imaging. Positron emitting radiopharmaceuticals are used to target malignant or metabolically active tissue[16]. PET was considered a research tool when first applied to oncology by Di Chiro *et al.*[17] for the characterization of brain tumours. The application of PET to colorectal cancer dates back to 1982[18] and is based on the finding that the tracer 2-[^{18}F]fluoro-2-deoxy-D-glucose (F-18-FDG) concentrates in malignant tissue owing to an increase in glycolysis compared with normal tissue[19,20]. Conventional imaging studies, such as CT, MRI and ultrasonography, rely on structural change within the tissue and are reasonably good methods for assessing local infiltration in advanced tumours[12,21,22]. Functional imaging, such as PET and immunoscintigraphy, rely on abnormal cellular metabolic activity that usually precedes any structural change[23,24]. In the context of colorectal cancer, the areas of weakness described for conventional imaging are the areas of strength for PET[25–27]. This article outlines the biological basis and technical considerations for the use of F-18-FDG PET in colorectal cancer, and examines the experience gained to date with F-18-FDG PET in colorectal cancer management.

Methods

A review of the literature was undertaken by searching the Medline database for the period 1980–2000. Keywords used were: 'PET', 'positron emission tomography', 'colorectal cancer', 'treatment evaluation', 'staging' and 'computed tomography'. Articles retrieved were checked manually and only those references that were of relevance or contained validated new information were included.

Rationale for the use of positron emission tomography in the management of colorectal cancer

Biological basis

The biological basis for PET centres on the observations made by Warburg[28,29] that cancer cells exhibit enhanced glycolysis. Glucose is preferentially concentrated in malignant cells owing to an increase in membrane glucose transporters[30,31] as well as an increase in some of the principal enzymes, such as hexokinase, phosphofructokinase and pyruvate dehydrogenase[31–33].

F-18-FDG, a radiolabelled analogue of glucose, decays by positron emission and is a commonly used tracer in PET imaging. Like unlabelled glucose, it concentrates in malignant cells. As it decays a positron is emitted which, after a very short path, collides with an electron. This leads to the release of two photons of 511-KeV energy, which travel at almost 180° to each other[16]. A PET scanner is designed to detect these photons and determine their point of origin, such that an image can be constructed based on detected activity. The mechanism by which F-18-FDG accumulates in human cancer cells is based on an increase in the expression of glucose transporter molecules on the cell surface[34–36]. Activation of the gene coding for the synthesis of the glucose transporter, *GLUT1*, is a major early marker of cellular malignant transformation[37]. Once transported into the cell, F-18-FDG is phosphorylated to F-18-FDG-6-phosphate by hexokinase, but takes no further part in the glycolytic or glycogen synthetic pathway. F-18-FDG-6-phosphate cannot diffuse out of the cell and becomes metabolically trapped as accessory metabolic pathways are too slow for the half-life of F-18-FDG. Therefore, the detected activity of fluorine-18 relates closely to the accumulated F-18-FDG-6-phosphate and the glycolytic activity for exogenous glucose[38,39]. *Fig 1* illustrates this process schematically.

Although this model is widely accepted, there are certain important physiological factors that influence F-18-FDG uptake. These include tissue oxygenation, glucose utilization, regional blood flow and the inflammatory reaction surrounding the tumour[40–42]. The signal recorded from

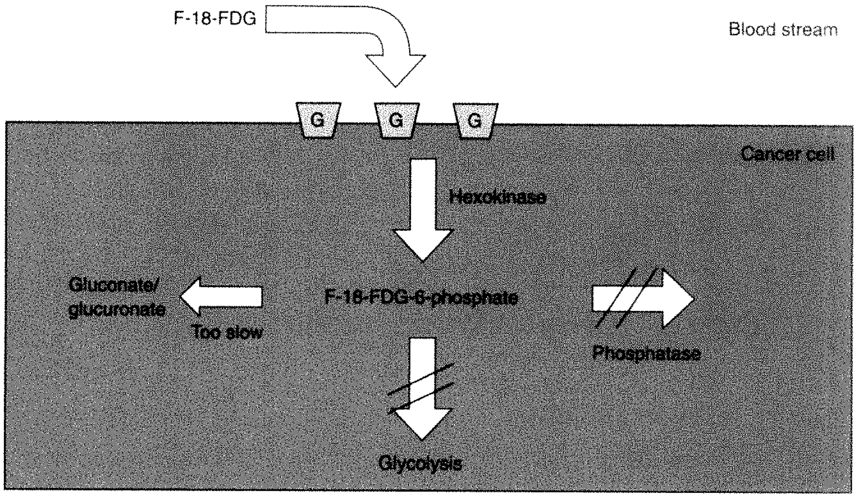


Fig. 1 2-[^{18}F]fluoro-2-deoxy-D-glucose (F-18-FDG) is transported into cancer cells as a result of upregulation of membrane glucose transporters (G). Once phosphorylated by hexokinase to F-18-FDG-6-phosphate, the fluorine-18 is metabolically trapped. F-18-FDG-6-phosphate takes no further part in glycolysis and there is little phosphatase activity. Alternative pathways to gluconate and glucuronate are too slow for the half-life of this compound and it cannot diffuse out of the cell

F-18-FDG may be from a composite area, interfering with the specificity of F-18-FDG PET for malignant tissue[41,42]. For example, there may be difficulty in differentiating tumour from active inflammation in the pelvis, as F-18-FDG uptake reflects the metabolic activity of a tissue[25,43,44].

Technical aspects

Instrumentation

The past 5 years have witnessed a significant expansion of the technology available for PET imaging. The originally designed, full ring, multicrystal, whole body tomographs remain the gold standard. Improvements in software have impacted on their utility and acceptance. These instruments are expensive, but set the standard to be achieved. They make use of special bismuth germanate oxide radiation detectors and detector assemblies. Manufacturers are trying to address the need for cheaper instrumentation, and a range of different types of PET scanner are now available. New radiation detector technology is coming on to the market (lutecium orthosilicate and other types), and the basic Anger γ camera with sodium iodide crystals has been adapted such that PET imaging can also be performed. However, many, if not most, of these proposed solutions represent a technological compromise; their ultimate place and role have yet to be determined.

Procedure

A detailed description is not appropriate in this review. Patients are scanned fasting in order to maximize F-18-FDG uptake. In anxious patients an anxiolytic/myorelaxant is given to minimize confounding F-18-FDG uptake in muscle. Administration of hyoscine butylbromide may reduce uptake in normal bowel, which is another potentially

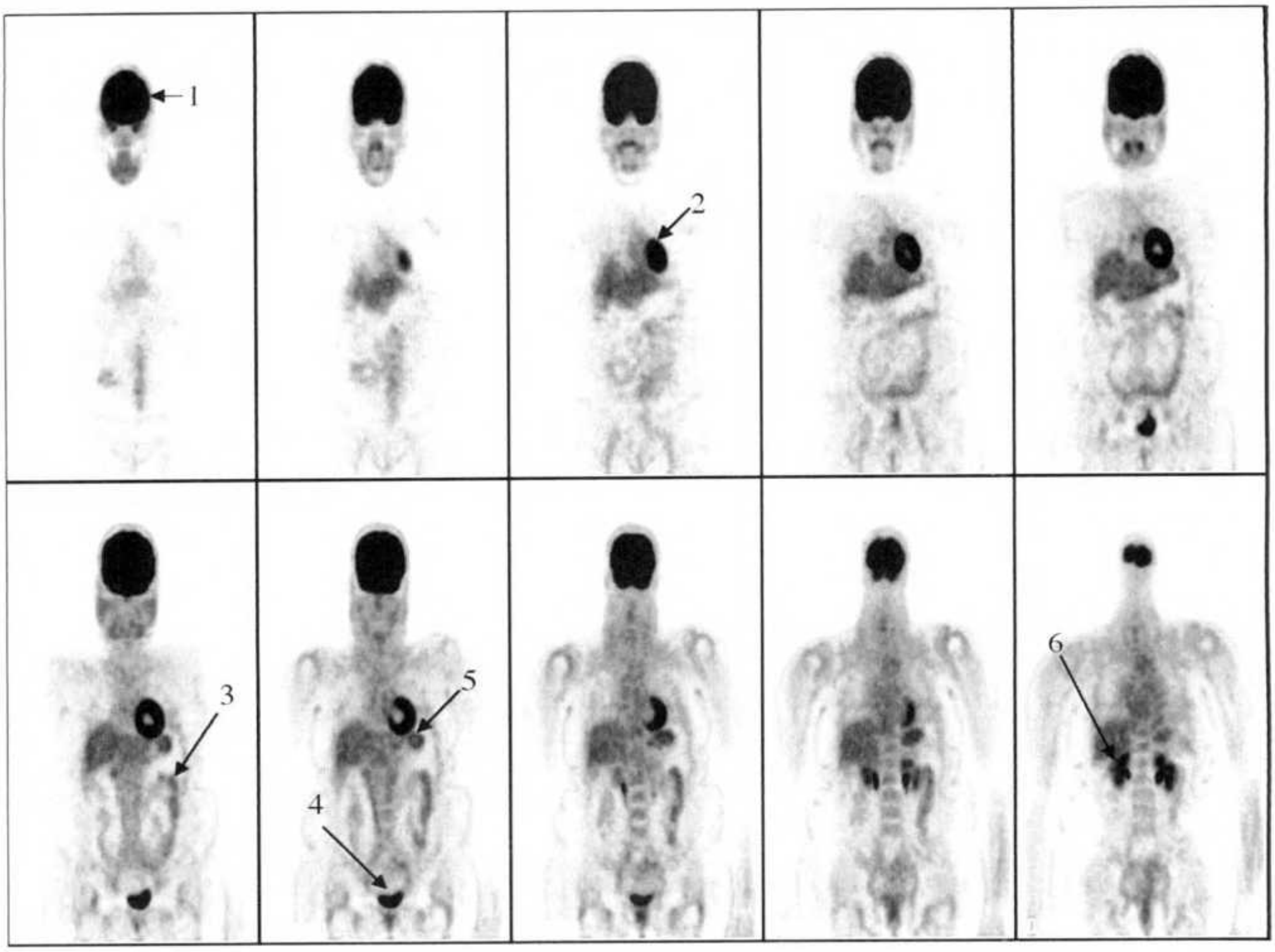


Fig. 2 Coronal sections of a normal 2-[^{18}F]fluoro-2-deoxy-D-glucose positron emission tomography study. The top left image is most anterior and subsequent images are more posterior until the final section is reached (bottom right). Normal uptake is seen in the brain (1), myocardium (2), colon (3), urinary bladder (4), stomach (5) and renal pelvis (6)

confounding factor. The scanning sequence can be tailor made, but scanning is usually optimized from the base of the skull to the upper third of the lower limb. Alternatively, whole body scans are recorded. Data can be obtained both from tracer emission and transmission data sets, for improved data analysis and quantification.

Image analysis

Whole body data can be analysed in terms of the pattern of distribution of labelled F-18-FDG and also with respect to the measurement of tracer uptake in a specific site. The quality of the data obtained depends significantly on the merits of the instrument used, the software available and the amount of tracer administered. A typical normal whole body scan for F-18-FDG is shown in *Fig. 2*. Normal and significant F-18-FDG uptake is always noted in the brain, and frequently, but variably, in the myocardium, stomach, liver, spleen, muscle and the lumen of the gastrointestinal tract. The bone marrow is highlighted, with variable intensity, often reflecting response to chemotherapy. The F-18-FDG tracer is eliminated by the kidneys, which are depicted along with the urinary bladder. Since the data are recorded in three dimensions, data sets can be formatted into any plane (transaxial, coronal and saggital).

F-18-FDG regional uptake is expressed in terms of semiquantitative standardized uptake values or differential absorption ratios (DARs). The DAR is calculated as (PET count × calibration factor)/(injected dose [millicuries]/body-weight [kilograms])[45,46]. These indices are useful in distinguishing inflammatory from neoplastic tissue uptake, and for monitoring disease *versus* time and/or therapy, but significant spread of this type of data must be taken into account. The use of a minimum DAR of 3 to diagnose malignant tissue, derived from PET studies of patients with colorectal cancer, may help to increase the specificity for this disease[47]. This method of analysing F-18-FDG accumulation has, however, been criticized[48] and normal gastrointestinal uptake with DAR values of 5–10 in the stomach, liver and intestine has been reported[49]. The accuracy of PET may be further enhanced by using external markers in order to compare conventional imaging with those acquired using F-18-FDG uptake. *Figs 3–5* are examples of PET images from patients with colorectal cancer studied at this institution. *Fig. 6* is a CT image of the patient presented in *Fig. 5*.

Possible applications of positron emission tomography in the management of colorectal cancer

Screening and diagnosis of primary colorectal cancer

Programmes of screening for colorectal cancer are described elsewhere[50] but evaluations of PET in this context are scarce. The availability and cost of PET (a PET study

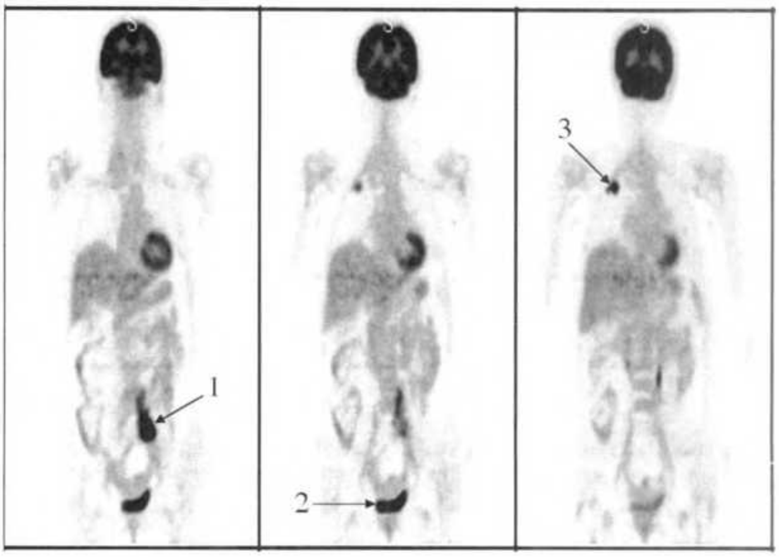
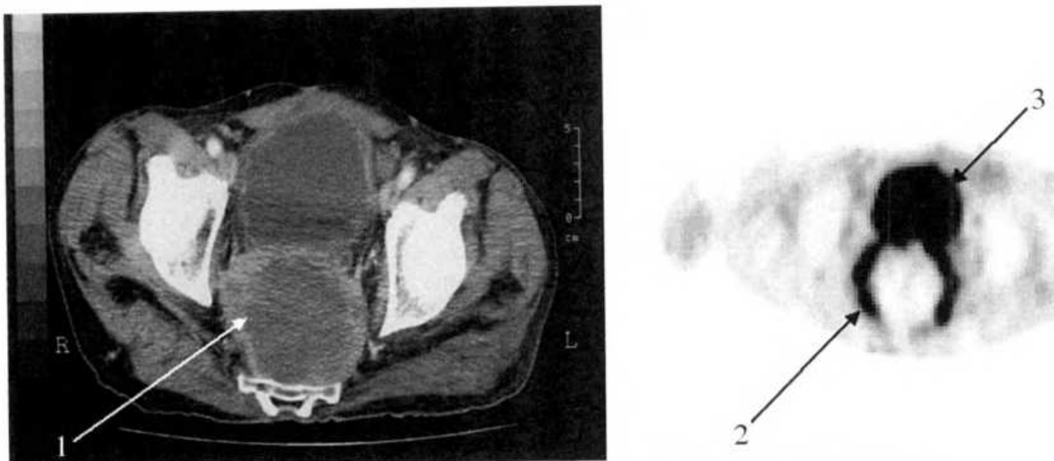


Fig. 3 Three coronal sections of a 2-[^{18}F]fluoro-2-deoxy-D-glucose (F-18-FDG) positron emission tomography study in a patient who underwent a Hartmann's procedure for a sigmoid adenocarcinoma followed by reversal. Sections are from anterior to posterior going from left to right. Increased F-18-FDG uptake representing local recurrence is seen in the region of the anastomosis (1). There is normal uptake in the urinary bladder (2). In addition, marked F-18-FDG uptake is seen in the upper zone of the right lung (3) representing a pulmonary metastasis. Computed tomography of the abdomen and pelvis was reported as showing benign fibrotic tissue at the anastomosis



a Computed tomography **b** Positron emission tomography

Fig. 4 **a** Computed tomography through the pelvis showing a large pelvic recurrence (1) in a patient who had previously undergone abdominoperineal resection for adenocarcinoma of the rectum. **b** Transaxial 2-[^{18}F]fluoro-2-deoxy-D-glucose positron emission tomography image confirms circumferential recurrence with central necrosis (2) behind the urinary bladder (3)

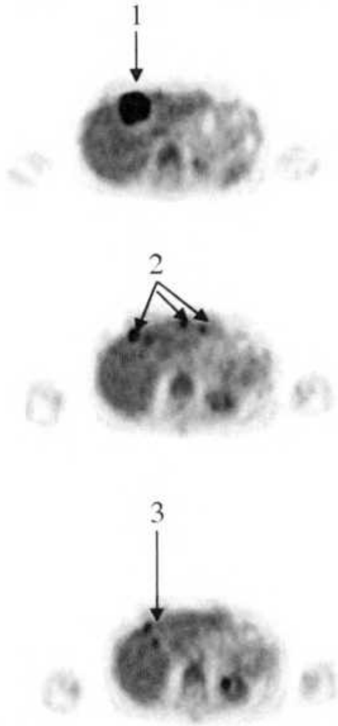


Fig. 5 2-[^{18}F]fluoro-2-deoxy-D-glucose positron emission tomography study showing a large metastasis (1) in the liver of a patient who had previously undergone colonic resection for adenocarcinoma. This is also evident in *Fig. 6*. In lower transaxial sections there are multiple lesions (2 and 3) which are not apparent on the computed tomography image shown as *Fig. 6*

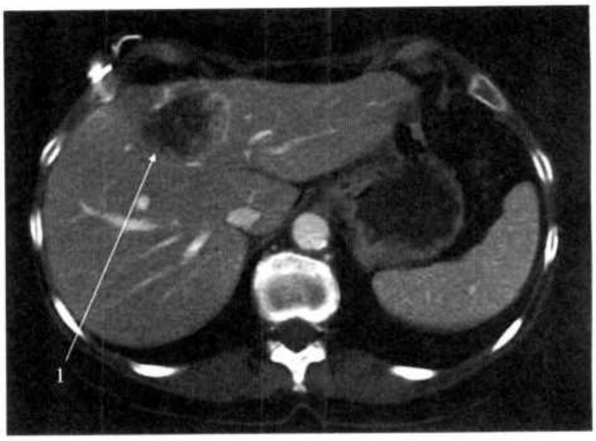


Fig. 6 Spiral computed tomography (CT) image at the level of the liver in a patient with an apparently solitary liver metastasis (1). Although no other abnormal lesions were detected on CT, 2-[^{18}F]fluoro-2-deoxy-D-glucose positron emission tomography (*Fig. 5*) clearly demonstrates multiple lesions

currently costs approximately twice as much as CT of the abdomen and pelvis[51]) are two important contributory factors. However, diagnosis of colorectal cancer in symptomatic individuals is possible with PET[45,52]. Ruhlmann *et al.*[53] described one patient diagnosed confidently with PET and confirmed the absence of tumour in two patients. Takeuchi *et al.*[45] confirmed the diagnosis in six patients with histologically proven colorectal cancer. Both reports were part of larger studies of the use of PET for imaging recurrent colorectal cancer. There are more published data on PET for recurrent cancer than on PET for primary cancer.

Currently, endoscopy and barium enema detect over 90 per cent of colorectal cancers[54], and are cheaper and more readily available than PET. Another technique, hydrocolonic sonography, is very accurate and has the potential to become a diagnostic tool[55]. CT pneumocolon is an emerging technique that is minimally invasive, sensitive and specific for colorectal cancer[56,57]; it has a low morbidity and is cost effective. The application of three-dimensional CT reconstruction allows inspection of the mucosa and the possibility of 'virtual' colonoscopy[58,59]. This technique, however, requires further refinement[60]. These factors mean that PET is unlikely to play a significant role in diagnosing primary colorectal cancer at present, although its use as a screening investigation has yet to be fully evaluated. However, Rigo *et al.*[61] have stated that on current evidence inclusion of patients with low disease probability increases the likelihood of false-positive PET scans; this renders the technique unsuitable for unselected screening programmes.

Staging primary colorectal cancer

The aim of preoperative staging investigations is to identify the extent of local infiltration, involvement of lymph nodes and metastases to the liver. Accurate staging allows planning of any surgical procedure and informed decision making on additional treatments, for example neoadjuvant radiotherapy[62,63]. Liver metastases are found in 10–25 per cent of patients at the time of the initial operation for primary colorectal cancer. Of these patients 25 per cent are candidates for resection[64].

Local infiltration

CT is accepted as an accurate method of assessing local infiltration, especially in advanced tumours. Sensitivities of 61, 55 and over 70 per cent have been reported[11,12,65]. The accuracy of CT and MRI for detection and characterization of transmural penetration in low-stage tumours is poor. The development of more sophisticated CT, MRI and endoluminal MRI coils will affect these results[65]. Endoanal ultrasonography provides useful clinical evaluation of the depth of invasion of rectal cancers[21,66–70]. Laparoscopy allows direct visualization of the peritoneal surface and laparoscopic ultrasonography may further improve staging accuracy[71], but this is invasive, requires a general anaesthetic and information that could alter treatment is not available before operation.

Involvement of lymph nodes and extrahepatic intra-abdominal spread

Standard evaluation of lymph nodes and extrahepatic, intra-abdominal spread with CT and MRI is poor[12,72–74]; reported accuracy for CT ranges from 25 to 73 per cent with a sensitivity of 40 per cent for MRI. Overall sensitivity for malignant lymphadenopathy detected by CT is approximately 45 per cent[7]. Immunoscintigraphy has been shown by some to be significantly more sensitive for pelvic (74 per cent) and intra-abdominal (66 per cent) disease than CT (57 and 34 per cent respectively)[75], but other reports have noted a sensitivity of 23 per cent, a positive predictive value of 33 per cent and a negative predictive value of 37 per cent[76] for this technique.

Metastases to the liver

In the USA approximately 14 000 patients per year present with isolated liver metastases as their first recurrence[77] and about 20 per cent of these patients die with metastases exclusively in the liver. Ferrucci[78] suggests that the following questions should be answered when investigating the liver. (1) Are tumours present? (2) Are all lesions visible? (3) Are they malignant? (4) Are they resectable? The sensitivity of transabdominal ultrasonography is too low to exclude small lesions in the liver[50]. CT has a reported sensitivity of 72 per cent and specificity of 99 per cent; MRI yields similar results[12,79]. However, CT fails to demonstrate lesions in 7 per cent of cases and underestimates the number of lobes involved in 33 per cent[78,80]. Intraoperative ultrasonography is a sensitive investigation and is possible laparoscopically, but its use is not universal. Immunoscintigraphy has shown promise in this aspect of staging[81–84], with liver metastases not evident on CT being detected using indium-111-labelled anti-CEA antibodies. Although the sensitivity of immunoscintigraphy is high, specificity can be a problem, along with poor spatial resolution when compared with F-18-FDG PET.

Role of positron emission tomography

Studies by Falk *et al.*[51], Abdel-Nabi and colleagues[85], Ogunbiyi *et al.*[26] and Mukai and co-workers[52] form the most significant contribution to the debate on the use of F-18-FDG PET in the staging of primary colorectal cancer. None of these studies comments on the ability of F-18-FDG PET to stage local infiltration, but it should be noted that synchronous tumours can be detected if present. Falk's study showed, in a cohort of 16 patients with 15 cancer foci (12 colorectal, two liver and one mesenteric), that F-18-FDG PET was superior to CT for staging (positive predictive value 93 per cent and negative predictive value 50 per cent for F-18-FDG PET *versus* 100 and 27 per cent respectively for CT). The group contained patients with primary and recurrent colorectal cancer and, interestingly, one liver (2–3 mm in size) and one mesenteric metastasis were missed by F-18-FDG PET. In a more recent study, Abdel-Nabi *et al.*[85] investigated 48 patients with colorectal cancer (44 with biopsy-proven disease and four with highly suspicious findings). F-18-FDG PET had a positive predictive value of 90 per cent and a negative predictive value of 100 per cent, but the sensitivity for lymph node metastases was 29 per cent, similar to that of CT. Detection of liver metastases by F-18-FDG PET was superior to that of CT (sensitivity 88 *versus* 38 per cent), but this was in only eight patients. So there appears to be a place for F-18-FDG PET in staging primary colorectal cancer and PET has the advantage of studying the whole body, thus requiring the patient to be submitted to a single staging investigation. F-18-FDG PET has been shown to be accurate for the detection of metastases to the liver in a small study group, but its accuracy for the detection of metastatic lymph nodes[52] is not fully established; further evaluation of F-18-FDG PET for staging primary colorectal cancer is required.

Surveillance and detection of recurrent colorectal cancer in treated patients

In up to 30 per cent of cases, recurrence following treatment of primary colorectal cancer is localized and so suitable for curative resection[86]. If detected early, survival may be improved, especially if radical surgery is performed[87]. To avoid unnecessary morbidity and mortality appropriate selection of patients is essential, as only 20–30 per cent of these particular cancers are curable[88,89].

Sensitivity (59 per cent) and specificity (84 per cent) for recurrence with serial measurement of CEA is low[10]. Routine surveillance with CT has gained acceptance, and its sensitivity for detecting intra-abdominal and pelvic abnormalities is high. However, differentiating benign fibrosis from tumour recurrence is difficult, if not impossible, without the use of a metabolic signal. This is especially so in the pelvis, where CT is not sufficiently specific[6]. MRI may give additional information and technology continues to improve, but there have been reports questioning its ability to distinguish recurrent tumour from fibrosis[90–92]. Immunoscintigraphy with indium-111-labelled B72.3 (sensitivity 74 per cent)[93], technetium-99m-radiolabelled anti-CEA monoclonal antibody (sensitivity 91 per cent)[94] and technetium-99m-labelled BW431/26 (sensitivity 92 per cent)[95] all show promise in the detection of recurrent colorectal cancer; this technique appears superior to CT for differentiating scar from tumour in the pelvis[96].

A dilemma also exists in the management of patients who present with an isolated increase in CEA level, with minimal symptoms and normal imaging. Observation may result in losing the opportunity to resect a curable recurrence, but aggressive investigation leads to psychological and physical morbidity, as well as incurring significant cost. In this context, tissue biopsy has a false-negative rate due to sampling error[97], even with CT guidance. Second-look laparotomy can lead to a definite diagnosis in 90 per cent of cases, yet between 12 and 60 per cent of these patients are unsuitable for resection[3] and suffer substantial morbidity. These findings contribute to the ongoing debate about the need for aggressive follow-up to detect recurrence after treatment for colorectal cancer[98].

Positron emission tomography versus *carcinoembryonic antigen measurement*

Flanagan *et al.*[99] assessed the potential role of F-18-FDG PET in patients with an unexplained increase in CEA level and normal CT scans. In 17 of 22 patients PET was abnormal, and tissue sampling and/or follow-up confirmed recurrence. Biopsy was performed in seven patients of whom four underwent curative surgical intervention. The clinical course and radiological follow-up confirmed extensive disease in eight of ten patients who did not undergo biopsy or operation. PET had a positive predictive value of 89 per cent and a negative predictive value of 100 per cent. While these results are encouraging, they should be interpreted cautiously as the study was small with the possibility of bias associated with retrospective analysis. The follow-up period of 6 months with CT or MRI to confirm a false-positive PET scan is probably insufficient, based on knowledge of the natural history of colorectal cancer. Finally, the method of analysis used to calculate F-18-FDG accumulation has been criticized and a more rigorous calculation of actual glucose metabolism suggested[48]. The superior accuracy of PET over CEA measurement for the detection of recurrent disease has also been discussed by Schiepers *et al.*[27], Takeuchi and colleagues[45], Delbeke and co-workers[43], and Beets and colleagues[100].

Positron emission tomography versus *conventional imaging*

Strauss and colleagues[25], Schlag *et al.*[44], Ito *et al.*[15] and Schiepers and colleagues[27] have all confirmed the value of F-18-FDG PET in differentiating scar tissue from local recurrence (*Table 1*). In Schiepers' study[27], 76 patients with confirmed or suspected recurrence were evaluated with F-18-FDG PET *versus* routine imaging (CT, ultrasonography, plain radiography). Sensitivity for disease in the pelvis was 93 per cent with F-18-FDG PET against 60 per cent with CT. The study also emphasized the value of F-18-FDG PET in detecting both hepatic disease (sensitivity 94 per cent with PET *versus* 85 per cent with CT/ultrasonography) and extrahepatic disease (14 foci in ten patients). Takeuchi *et al.*[45] also reported the accuracy of F-18-FDG PET in the diagnosis of pelvic recurrence in 15 of 16 patients with histologically proven pelvic recurrence (four of these patients had equivocal CT or MRI). These authors stated, by retrospectively analysing the DAR values and clinical findings, that a DAR cut-off value of 2·8 would have resulted in diagnosis with 100 per cent accuracy. It is also noteworthy that the CEA level was normal in five patients with recurrent lesions detected on F-18-FDG PET.

Table 1 Studies of positron emission tomography in the detection and staging of recurrent colorectal cancer

			Sensitivity (%)		Specificity (%)	
Reference	Year	No. of patients	PET	CT	PET	CT
Yonekura *et al.*[18]	1982	3	100		100	
Strauss *et al.*[25]	1989	29	100		100	
Gupta *et al.*[101]	1991	18	100	70	86	43
Ito *et al.*[15]	1992	15	100		100	
Gupta *et al.*[102]	1993	16	90	60	66	100
Larson *et al.*[103]	1994	59	93		78	
Beets *et al.*[100]	1994	35	100 Local			
			47 Liver			
Pounds *et al.*[104]	1995	57	95		87	
Schiepers *et al.*[27]	1995	76	93 Local	60	97	72
			94 Liver	85	100	
Daenen *et al.*[105]	1996	19	95		87	
Lai *et al.*[106]	1996	34	93 Liver	100	100	100
			100 Non-liver			
Vitola *et al.*[107]	1996	24	90	86	100	100
Delbeke *et al.*[43]	1997	52	91	81	96	60
				97*		5*
Ruhlmann *et al.*[53]	1997	59	100		67	
Ogunbiyi *et al.*[26]	1997	40	91 Local	52	100	80
			95 Liver	74	100	85
Valk *et al.*[108]	1999	115	93	69	98	96
Flanagan *et al.*[99]	1998	22	100		71	
Takeuchi *et al.*[45]	1999	23	94		100	

*With computed tomographic portography. PET, positron emission tomography; CT, computed tomography

Staging of recurrent colorectal cancer

Current investigations are not sufficiently sensitive to differentiate isolated resectable disease from disseminated metastases. Reported sites most commonly affected by metastases vary. One study has reported 13 per cent to the liver, 4 per cent to abdominal lymph nodes, 3 per cent to lung, 2 per cent peritoneal, 1 per cent to bone and 1 per cent to brain[78]. Another suggests that the liver is affected in 33 per cent of cases, lung in 22 per cent, locoregional sites in 21 per cent, intra-abdominal sites in 18 per cent, retroperitoneal sites in 10 per cent and intraluminal sites in 6 per cent[5]. PET offers a highly sensitive, non-invasive means by which to detect recurrence and select the most appropriate treatment. Early reports suggest that F-18-FDG PET is more accurate than CT for the purpose of staging recurrent colorectal cancer[27,100].

Hepatic metastases

Beets *et al.*[100] demonstrated the value of whole body F-18-FDG PET for detecting colorectal cancer metastases to the liver. Schiepers et al.[27] confirmed a higher sensitivity and accuracy with F-18-FDG PET (94 and 98 per cent respectively) compared with CT/ultrasonography (85 and 93 per cent) in the detection of hepatic metastases. There were no false positives for F-18-FDG PET and one for CT/ultrasonography. There were two false-negative F-18-FDG PET scans for two nodules discovered in the liver at surgery. In each of the two patients the nodule was less than 1 cm in diameter and not detected by CT. Delbeke *et al.*[43] compared F-18-FDG PET with CT and CT portography; 52 patients were evaluated on 61 occasions (portography was not performed in all patients). The final diagnosis was made by histopathology in 44 patients and clinical/radiological follow-up in the remainder. For the 127 hepatic lesions identified in these patients, F-18-FDG PET had a sensitivity of 91 per cent, compared with 81 and 97 per cent for CT and CT portography respectively. For the 39 extrahepatic lesions the sensitivity of PET was 100 per cent, compared with 74 per cent for CT. Ogunbiyi *et al.*[26] assessed 40 patients with recurrent cancer and reported a higher sensitivity (95 per cent) and specificity (100 per cent) for F-18-FDG PET than for CT (74 and 85 per cent respectively) in the detection of hepatic metastases. These authors also found a higher accuracy for PET than for CT in delineating multiple liver lesions. More recent studies have demonstrated the value of F-18-FDG PET in the detection of both hepatic and extrahepatic metastases from colorectal cancer[43,107].

Extrahepatic metastases

Lai *et al.*[106] compared F-18-FDG PET with conventional imaging in 34 patients (CT in 34 with chest CT in 19 and chest radiography in 15). Unsuspected extrahepatic disease was confirmed in 11 patients and clinical management was affected in ten. Schiepers *et al.*[27] also reported that a significant number of unexpected extrahepatic metastases could be demonstrated using F-18-FDG PET. In this study, a total of 14 of 25 abnormal foci detected with F-18-FDG PET were confirmed by biopsy as metastases; the 11 false positives were all in the thorax. With image correlation and careful patient selection the false-positive rate may be reduced.

These studies demonstrate that PET is highly sensitive and accurate in the diagnosis and staging of recurrent colorectal cancer. Detection of unsuspected metastases by F-18-FDG PET ranges from 6 to 32 per cent[43,106] (*Table 2*). A multicentre study by the Institute of Clinical PET examined the cost effectiveness of 14 PET centres (reviewing the records of 267 patients who had been treated for

Table 2 Studies demonstrating that positron emission tomography detects colorectal cancer metastases unsuspected on conventional imaging

Reference	Year	No. of patients	Unsuspected metastases
Schiepers *et al.*[27]	1995	76	10 of 76 (13)
Lai *et al.*[106]	1996	34	11 of 34 (32)
Delbeke *et al.*[43]	1997	52	3 of 52 (6)
Valk *et al.*[108]	1999	115	23 of 78 (29)

Values in parentheses are percentages

colorectal cancer) and found that a potentially large saving could be made if F-18-FDG PET were to be incorporated into the routine management of recurrent disease. This is based on the reduction of unnecessary laparotomies performed (from 20 to 10 per cent) and the increased number of resections with curative intent[103].

Evaluating treatment in patients with colorectal cancer

Accurate information about the antitumour effect of radiotherapy and/or chemotherapy for colorectal cancer would guide planning and ongoing adjuvant therapy. Nagata *et al.*[109] demonstrated a correlation between F-18-FDG uptake and tumour response in patients treated for primary or metastatic liver lesions. Studies assessing the uptake of F-18-FDG measured by PET and correlation with the antitumour effects of chemotherapy have been reported for certain tumour types, including colorectal cancer[110–114].

Radiotherapy

The use of PET was evaluated by Haberkorn *et al.*[115] in 21 patients (41 examinations) with recurrent disease before and after radiotherapy treatment. A correlation was made between palliative benefit and reduction in F-18-FDG uptake in half of the patients, and F-18-FDG PET was found to be more accurate than CEA in assessing response. The authors postulated that the inflammatory response to radiotherapy might have caused an increase in F-18-FDG accumulation, which was wrongly interpreted as residual tumour. They suggest that a delay in post-treatment F-18-FDG PET evaluation of 6 months might increase the accuracy in terms of assessing tumour response. These findings mirror the results of other reports, such as those of Abe *et al.*[116] and Engenhart *et al.*[117].

Chemotherapy

The situation for monitoring chemotherapy in advanced colorectal cancer appears more promising. Early reports have shown high concentrations of $[^{18}\text{F}]$5-fluorouracil (F-18-FU) in responsive tumours[118–120]. Strauss and Conti[121] studied PET images of F-18-FU metabolite concentration and compared these with growth rate (using volume measurements of metastases on CT) before and during chemotherapy. Lesions with low FU uptake had a significant increase in volume and no response to treatment. The pharmacokinetics of FU[122–124] suggest that F-18-FU may be superior to F-18-FDG in predicting the response to treatment on an individual patient basis. Recently, Findlay *et al.*[110] evaluated the metabolism of colorectal cancer liver metastases using F-18-FDG PET before and at intervals after treatment in 18 patients. The findings were compared with tumour outcome as assessed conventionally by change in size on CT. Results were expressed as a ratio of F-18-FDG uptake in tumour and in normal liver. This ratio 4–5 weeks after treatment was able to discriminate response from non-response, both in lesion-by-lesion and overall patient terms (sensitivity 100 per cent for both, specificity 90 and 75 per cent respectively).

In 1994 a meeting of the European Community PET Oncology Concerted Action Group and the European Organization for Research and Treatment of Cancer PET Study Group brought together many members of the 31 European PET centres[125]. Their collaboration with oncologists led to the study of tumour response to chemotherapy in 12 groups and to radiotherapy in three groups. Although there was wide variation in the methodology between centres, assessment of F-18-FDG uptake was thought to be a satisfactory method for functionally imaging tumours, assessing metabolic rate and providing information on response to treatment. Patients with the biggest reduction in F-18-FDG uptake showed the best clinical response, and the use of $[^{11}\text{C}]$thymidine or $[^{11}\text{C}]$amino acids as tracers may provide an even more specific marker of cellular proliferation. These conclusions, along with proposals to advance PET methodology, to run PET studies in parallel with phase I/II clinical trials and to collect more information regarding the application of PET to specific tumours, provide an indication of the expectation of the role of PET in the management of colorectal cancer.

Conclusion

The application of F-18-FDG PET to oncology, and in particular to the management of colorectal cancer, continues to gain acceptance. PET is an expensive modality; start up costs are high because of the need to establish tracer production and distribution, purchase hardware and software, and train staff. There are approximately 200 PET systems in clinical use in the USA where state-of-the-art PET systems cost from US $800 000 to 2·5 million. In the

UK, PET systems cost between £1 and 1·5 million. The overall cost of whole body F-18-FDG PET is approximately £800 per study, of which £350 is required for a single dose of F-18-FDG. The cost of spiral CT of the abdomen and pelvis is £350.

There is a significant body of evidence to favour the use of F-18-FDG PET in the assessment of recurrent colorectal cancer mainly because it leads to alteration in clinical management[43,106,108] (for example, surgery may be avoided owing to the identification of non-resectable tumour). Valk *et al.*[126] studied patients with lung cancer (99 patients), recurrent colorectal cancer (57), melanoma (36), and head and neck cancer (29). They found that the savings from contraindicated surgical procedures exceeded the cost of PET by ratios of 2 : 1 to 4 : 1, depending on the indication. This same group also studied 115 patients with recurrent colorectal cancer and demonstrated that the potential saving from the diagnosis of non-resectable tumour by PET was approximately US$3000 per preoperative study[108]. This work confirms significant cost savings, similar to those obtained when F-18-FDG PET is used to assess solitary lung nodules[127], which must be considered when assessing the true cost of PET. The role of F-18-FDG PET in differentiating scar tissue from recurrent colorectal cancer is well established and PET is increasingly important for monitoring chemotherapy. More work is required to evaluate the use of F-18-FDG PET for screening, detection and staging of primary colorectal cancer.

Ongoing and future developments

Significant progress is to be expected with PET and other cross-sectional imaging modalities. Instrumentation continues to develop and multimodality imaging, using a single instrument, is a reality, with CT and/or MRI combined PET scanners and γ cameras capable of PET coming on to the market. This should allow cross-sectional imaging techniques (CT/MRI), which offer detailed anatomical information, and functional imaging techniques (PET), which offer metabolic information, to come together to increase the accuracy of imaging. As with any radionuclide tracer technique, PET will evolve in parallel with tracer development. A number of new tracers are being developed; the fluorine-18-labelled thymidines in particular are promising.

To fulfil its full clinical potential, PET must become more available, with instrumentation of a similar standard to that of the best PET technology currently available. Core centres able to label and distribute tracers form a fundamental infrastructure that must also be developed, if the promise of PET is to be realized worldwide.

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Systematic review

Somatostatin and octreotide in the prevention of postoperative pancreatic complications and the treatment of enterocutaneous pancreatic fistulas: a systematic review of randomized controlled trials

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Background: The aim of this study was to evaluate, through systematic review, the effectiveness of somatostatin and octreotide in the prevention of postoperative pancreatic complications and the treatment of established enterocutaneous pancreatic fistulas.

Methods: Electronic databases, including Medline and EMBASE, were searched systematically by using keywords including 'somatostatin', 'octreotide', 'fistula' and 'randomiz(s)ed controlled trial'. In addition, citations of relevant primary and review articles were examined. Particular authors were contacted when necessary. Data on patient recruitment, intervention and outcome were extracted from the included trials and analysed.

Results: Use of somatostatin or octreotide for the prevention of postpancreatectomy complications, including pancreatic fistulas, was identified in 14 randomized controlled trials, including one abstract and one conference proceeding, involving a total of 1686 patients. Use of somatostatin or octreotide for the treatment of established enterocutaneous pancreatic fistulas was identified in ten trials involving a total of 301 patients. Significant heterogeneity was found among the identified trials with regard to the definition of fistula, dosage of octreotide, starting time and duration of the treatment, among other factors.

Conclusion: There was major disagreement between the studies on whether use of the drugs in question is of value in preventing postoperative complications. This analysis suggests that, in units where the postoperative fistula rate following pancreaticoduodenectomy for neoplasia and other pancreatic conditions exceeds 10 per cent, somatostatin or octreotide administered before operation may significantly reduce the rate of major postoperative complications, particularly pancreatic fistulas. The identified evidence also suggests that there may be a limited role for such drugs in the treatment of established postoperative enterocutaneous pancreatic fistulas. A major conclusion is that further clarification of the roles of these drugs is still required through large, high-quality, randomized trials.

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Introduction

Discovered in 1972, somatostatin is a naturally occurring tetradecapeptide with a wide spectrum of mainly inhibitory biological actions[1]. Within the gastrointestinal tract, somatostatin has been found in the pancreas, stomach, intestinal mucosa and mesenteric neurones. Because of its inhibitory actions, it has been used in the management of upper gastrointestinal haemorrhage, secretory diarrhoea, dumping syndrome and peptide-secreting tumours. Since the mid-1980s, somatostatin has been advocated as an adjuvant therapy in the conservative treatment of patients with enterocutaneous pancreatic fistula. However, its short half-life (1·1–3·0 min) mandates continuous administration. Long-acting somatostatin analogues, such as octreotide acetate (Sandostatin SMS 210-995; Novartis Pharmaceuticals, East Hanover, NJ, USA) and lanreotide, have been developed[2,3]. Octreotide has a biological half-life of 90–120 min and can be administered subcutaneously two or three times per day. Compared with somatostatin, it not only significantly reduces the secretions of the pancreas and stomach, but also has a more selective range of targets[4]. Both somatostatin and octreotide have been described as effective treatments for enteric fistulas in adults, with few significant short-term side-effects (mostly diarrhoea, flushing and abdominal pain)[5–7]. These preparations have, therefore, the potential benefit of cutting the cost of treatment and reducing the discomfort of patients by shortening the length of hospital stay. On the other hand, there are several negative aspects of using these drugs, such as high cost, no therapeutic effect on underlying diseases, painful injection and potential long-term side-effects.

Surgical operation remains the primary treatment for tumours of the pancreas and periampullary region, and for chronic pancreatitis with disabling pain. Pancreatic operations carry an average major complication rate of 40 per cent and a mean mortality rate of 10 per cent[8]. In particular, pancreatic fistula, occurring in 10–20 per cent of patients, has been a leading cause of morbidity and death after pancreaticoduodenectomy[9]. Other major complications following pancreatic operations include dehiscence of the pancreaticojejunal anastomosis, ascites, abscess formation and pancreatitis. These problems may be accompanied by respiratory and/or renal failure, haemorrhage, shock and sepsis syndromes[10]. Such complications are mainly related to risk factors that include surgical trauma, technical difficulties in performing the anastomosis, and tension at the anastomotic site. It is alleged that, compared with those suffering from pancreatic or periampullary tumours, patients with chronic pancreatitis have a lower risk of developing postoperative complications due to fibrotic pancreas parenchyma and reduced digestive secretions[11–13].

As many as 75–85 per cent of enterocutaneous pancreatic fistulas develop after operation. Other causes of such fistulas include radiotherapy, chemotherapy, trauma, and diseases such as abdominal abscess, neoplasm and Crohn's disease[14]. High-output pancreatic fistulas (more than 200 ml daily) result in significant loss of fluid, alteration in acid–base status and malnutrition. In addition, digestive enzymes, often of extreme pH, can cause inflammation and soft tissue or skin destruction along the fistula tract. These increase the risk of infection and haemorrhage, prolong the time required for spontaneous fistula closure and increase postoperative mortality rates[15]. Conservative management of enterocutaneous pancreatic fistula, including total parenteral nutrition, skin care and infection control, succeeds in a spontaneous closure rate of 24–73 per cent[16]. However, such treatment is often of long duration (2–3 months) and high cost, and is associated with considerable morbidity[14]. In cases where maximal medical treatment has failed, reoperation for fistula closure is required.

In theory, reduction of fistula output should promote the chances of spontaneous closure. This is usually achieved by restriction of oral intake, sometimes supplemented with nasogastric suction in order to rest the bowel and pancreas. Pharmacological attempts to decrease intestinal secretions involve using drugs such as loperamide, atropine or its related substance pirenzepine, and H_2 blockers such as omeprazole, although the effectiveness of such treatments has yet to be confirmed[17].

Since 1990 many groups have described favourable experiences using somatostatin or octreotide for the 'conservative' management of enterocutaneous pancreatic fistula. Administration of such drugs has been reported to result in rapid and significant reduction of fistula output (often within 24 h of treatment) and acceleration of fistula closure (from as early as day 2)[18]. Reported adverse effects of octreotide have been rare[19]. It has also been speculated that prophylactic somatostatin or octreotide reduces the complication rate following pancreatic operations. The large-scale multicentre double-blind randomized controlled trial published by Büchler *et al.*[20,21] has been followed by similar clinical trials.

Martineau *et al.*[16] reviewed the role of octreotide for the management of established enterocutaneous pancreatic fistulas by summarizing the results of four randomized trials and eight case series. They concluded that octreotide significantly decreases enterocutaneous pancreatic fistula output, but does not significantly improve the rate of spontaneous fistula closure. Furthermore, octreotide does not appear to reduce the output, or hasten the time or rate of closure, in fistulas of recent onset (less than 8 days). Recently, Berberat *et al.*[8] reviewed six randomized controlled trials and one open randomized trial assessing the efficacy of octreotide in preventing postoperative complications in patients who underwent major pancreatic operations. They concluded that prophylactic use of octreotide can significantly reduce the complication rate. However, a more recent study by Yeo *et al.*[22] failed to find any benefit when octreotide was administered prophylactically.

In this review, the authors have performed a systematic search for randomized controlled trials on the effect of somatostatin and octreotide in the prevention and treatment of postpancreatectomy complications and established enterocutaneous pancreatic fistulas.

Methods

Electronic databases Medline (January 1960 to March 2000 inclusive) and EMBASE (January 1980 to March 2000 inclusive) were searched using the keywords 'somatostatin', 'octreotide', 'fistula' and 'randomiz(s)ed controlled trials' (search strategy: ['somatostatin' (explode) or 'octreotide' (explode) or 'fistula' (explode)] and ['randomised controlled trial' (explode) or 'randomized controlled trial' (explode)]). In addition, citations of relevant primary and review articles, and recent major conference proceedings, were examined and pursued. Particular authors were contacted when necessary.

Owing to the significant heterogeneity found among the identified trials, only results obtained from trials under similar conditions were pooled. An effort was made to classify the reported patients into high- and low-risk groups for pancreatic leakage. The high-risk group mainly included patients with various types of pancreatic cancer within an otherwise normally secretory pancreas; the low-risk group mainly included patients with chronic pancreatitis and a fibrosed pancreas.

Results

Preventive use of somatostatin or octreotide for major complications following pancreatic operations

Fourteen relevant randomized controlled trials including one abstract and one conference proceeding were retrieved[10,20,22–33], involving a total of 1686 patients (*Table 1*). One trial[33] (24 patients) assessed the effect of somatostatin on basal and stimulated exocrine pancreatic secretions after pancreaticoduodenectomy. Somatostatin treatment resulted in a significant reduction in the volume of drainage and in serum levels of amylase, lipase, protein and bicarbonate for 2 days. In addition, serum lipase concentration was reduced significantly for 5 days. The somatostatin group also had reduced pancreatic exocrine function, inhibited secretion of insulin and glucagon, and an earlier resumption of positive nitrogen balance (postoperative day 2 *versus* day 4 in the control group).

Two trials[31,32] (42 patients) studied the effectiveness of octreotide in the reduction of complications following pancreatic or pancreaticorenal transplantation. While both trials found no benefit for octreotide on graft survival, they reached different conclusions about the value of octreotide in the reduction of postoperative complications. Notably, the negative conclusion was derived from the trial where octreotide was administered after operation[31].

In three other trials[23,25,28], somatostatin or octreotide were also administered after the operation (*Table 2*). One trial[25] (35 patients), presented briefly by Beguiristain *et al.*, suggested a favourable effect of somatostatin in reducing the rates of pancreatic fistula formation ($P < 0.05$) and hastening closure ($P < 0.05$). The other two trials[23,28] (126 patients), however, largely denied any benefit for somatostatin or octreotide in reducing the overall postoperative complication rate or pancreatic drainage.

A recent large multicentre trial, conducted by Fourtanier *et al.*[30], reported results from 230 patients (including 30 with chronic pancreatitis). Here octreotide was administered during operation after random patient assignment was made in the operating room. Although a lower postoperative complication rate (22 per cent) was observed compared with that in the placebo group (32 per cent), the difference ($P < 0.08$) was not statistically significant. Similarly, no significant reduction in the rates of pancreatic fistula was observed in either of the stratified high- and low-risk groups (see Methods) (*Table 2*).

Among seven trials[10,20,22,24,26,27,29] that assessed the effectiveness of administering octreotide before operation, five (997 patients) reported a significantly reduced rate of pancreatic fistula formation (or drainage) with octreotide (*Table 3*). In terms of reduction of postoperative complication rates, two trials[27,29] (252 patients, including 23 with chronic pancreatitis) reported significant benefit with octreotide. For the remaining five trials it was possible to split the results according to the definition of high- or low-risk patients. Among these, two of four[10,20,22,24] involving high-risk patients reported a significant reduction of postoperative complications with octreotide (significant results in 301 patients *versus* non-significant results in 210), while two of four[10,20,22,26] involving low-risk patients reported a significant reduction of postoperative complications (significant results in 337 patients *versus* non-significant results in 129).

Therapeutic use of somatostatin or octreotide in the treatment of established enterocutaneous pancreatic fistula

In addition to the four randomized controlled trials of octreotide summarized by Martineau *et al.*[16], a further six trials were identified from the Medline and EMBASE search. All identified trials were published before 1996 (*Table 4*).

Distinct from the rest, the trial published by Gerardo Perez and Bernardo Acosta[40] compared 19 octreotide-treated patients with 50 'randomly' selected previous patients treated by conventional means. Their results suggested significant reductions in both fistula output and time required for closure. Another trial (45 patients), published by Isenmann *et al.*[41], deduced a significantly improved closure rate (90 per cent *versus* 20 per cent in the control group by day 30) with reduced closure time.

Among the remaining eight trials, four of six[34,36–39,42] reported significantly reduced fistula output with somatostatin or octreotide (significant results in 86 patients *versus* non-significant results in 50). In terms of fistula closure, none of the four trials[37,38,42,43] (175 patients) comparing

Table 1 Use of octreotide or somatostatin in preventing major complications following pancreatic operation

Reference	Year	Trial type	Recruit	Method	Follow-up	Treatment	Morbidity (trial group *versus* control)	Mortality (trial group *versus* control)
Büchler *et al.*[20]	1992	MC, RC, DB	246	OC group: 125 (68 high-risk, 57 low-risk) Control: 121 (71 high-risk, 50 low-risk)	90 postop. days	OC 100 μg/8 h for 7 days starting > 1 h preop.	32 *v* 55% (*P* < 0·005) High-risk group: 38 *v* 65% (*P* < 0·01) Low-risk group: 25 *v* 42% (n.s.) PF: 18 *v* 38% (*P* < 0·05)	3 *v* 6% (n.s.)
Buccoliero *et al.*[23]	1992	RC	16	SOM group: 8 Control: 8 All high-risk	—	SOM 250 μg/h for 6 days starting after operation	2 *v* 3 (n.s.). SOM group had lower amounts of drainage and lower serum amylase (*P* < 0·05)	—
Lange *et al.*[24]	1992	RC	21	OC group: 10 Control: 11 All high-risk	Up to 65 postop. days	OC 50 μg/8 h × 3, 100 μg/8 h × 3, thereafter 150 μg/8 h starting postop.	Problematic pancreatic drainage: 20 *v* 36% (n.s.) Gallbladder sludge: 100 *v* 50% (n.s.)	No death occurred
Pederzoli *et al.*[10]	1994	MC, RC, DB	252	OC group: 122 (76 high-risk, 46 low-risk) Control: 130 (86 high-risk, 44 low-risk)	90 postop. days	OC 100 μg/8 h for 7 days starting > 1 h preop.	16 *v* 29% (*P* = 0·01) High-risk group: 22 *v* 35% (n.s.) Low-risk group: 4 *v* 18% (*P* = 0·05) PF: 9 *v* 19% (*P* < 0·05)	3 *v* 6% (n.s.)
Beguiristain *et al.*[25]	1995	RC	35	SOM group: 21 (1 CP) Control: 14 (2 CP)	—	SOM 250 μg/h for 7 days starting postop.	PF: 10 *v* 36% (*P* < 0·05) Closure time: 5 *v* 19 days (*P* < 0·05)	Similar in two groups
Friess *et al.*[26]	1995	MC, RC, DB	247	OC group: 122 Control: 125 All low-risk	90 postop. days	OC 100 μg/8 h for 8 days starting > 1 h preop.	16 *v* 30% (*P* < 0·007) PF: 10 *v* 22% (*P* < 0·05)	2 *v* 1% (n.s.)
Montorsi *et al.*[27]	1995	MC, PRC, DB	218	OC group: 111 (8 CP) Control: 107 (10 CP)	60 postop. days	OC 100 μg/8 h for 7 days starting > 1 h preop.	22 *v* 36% (*P* < 0·05) PF: 9 *v* 20% (*P* < 0·05)	8 *v* 6% (n.s.)

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Table 1 Continued

Reference	Year	Trial type	Recruit	Method	Follow-up	Treatment	Morbidity (trial group *versus* control)	Mortality (trial group *versus* control)
Lowy *et al.*[28]	1997	PRC	110	OC group: 57 Control: 53 All high-risk	30 postop. days	OC 150 μg/8 h for 5 days starting postop.	30 *v* 25% (n.s.) Pancreatic anastomotic leak: 12 *v* 6% (n.s.)	1 death in OC group (n.s.)
Briceno Delgado *et al.*[29]	1998	PRC	34	OC group: 16 (2 CP) Control: 18 (3 CP)	—	OC 100 μg/8 h for 7 days starting > 1 h preop.	2 *v* 16 ($P < 0.05$) PF: 0 *v* 28% ($P = 0.03$)	1 death in control group (n.s.)
Fourtanier *et al.*[30]	1998	PRC, MC, DB	230	OC group: 122 (104 high-risk, 18 low-risk) Control: 108 (96 high-risk, 12 low-risk)	Up to 134 days	OC 100 μg/8 h for 10 days starting during operation	22 *v* 32% ($P = 0.08$) High-risk group: 23 *v* 33% (n.s.) Low-risk group: 17 *v* 25% (n.s.) PF: 17 *v* 19% (n.s.)	12 *v* 7% (n.s.)
Yeo *et al.*[22]	2000	PRC, DB	211	OC group: 104 (91 high-risk, 13 low-risk) Control: 107 (98 high-risk, 9 low-risk)	Up to 24 postop. months	OC 250 μg/8 h for 7 days starting within 2 h preop.	40 *v* 34% (n.s.) PF: 11 *v* 9% (n.s.) Mean hospital stay: 9 days in both groups	0 *v* 1% (n.s.)
Stratta *et al.*[31]	1993	PRC	25	OC group: 13 (9 PKT, 4 PT) Control: 12 (9 PKT, 3 PT)	10 postop. months	OC 100 μg twice daily for mean(s.d.) 8(4) days starting postop.	Similar in two groups Graft survival: similar in two groups	No death occurred
Benedetti *et al.*[32]	1998	PRC	17	OC group: 10 (8 PKT, 2 PT) Control: 7 (6 PKT, 1 PT)	6 postop. months	OC 100 μg/8 h for 5 days starting preop.	0 *v* 5 ($P < 0.05$) Graft survival (6 months): 90 *v* 86% (n.s.)	1 death in control group (n.s.)
Klempa *et al.*[33]	1991	RC	24	SOM group: 12 Control group: 12 All high-risk	—	SOM 250 μg/h for 5 days starting at laparotomy	SOM group had decreased levels of serum amylase (2 days) and lipase (5 days)	—

CP, chronic pancreatitis; DB, double blind; MC, multicentre; n.s., not significant; OC, octreotide; PF, pancreatic fistula; PKT, pancreas–kidney transplantation; PT, pancreas transplantation; (P)RC, (prospective) randomized controlled; SOM, somatostatin; *v*, *versus*

Table 2 Reduction of postoperative complications by administration of somatostatin or octreotide during or after operation

			Reduction of postoperative complications		
Reference	Year	Recruit	High-risk group	Low-risk group	Pancreatic fistula
Buccoliero *et al.*[23]	1992	16	16 (n.s.)	0	Reduced drainage and amylase ($P<0.05$)
Beguiristain *et al.*[25]	1995	35 (including 3 with CP)	—	—	Reduced formation and closure time ($P<0.05$)
Lowy *et al.*[28]	1997	110	110 (n.s.)	0	Reduced anastomosis leaks (n.s.)
Fourtanier *et al.*[30]	1998	230	200 (n.s.)	30 (n.s.)	Overall: 17 *versus* 19% (n.s.)

CP, chronic pancreatitis; n.s., not significant

Table 3 Reduction of postoperative complications by administration of somatostatin or octreotide before operation

				Reduction of postoperative complications			
Reference	Year	Type of study	Recruit	Mixed group	High-risk group	Low-risk group	Pancreatic fistula
Büchler *et al.*[20]	1992	MC, DB	246	—	139 ($P<0.05$)	107 (n.s.)	246 ($P<0.05$)
Lange *et al.*[24]	1992		21	—	21 (n.s.)	0	Reduced drainage (n.s.)
Pederzoli *et al.*[10]	1994	MC, DB	252	—	162 ($P<0.05$)	90 ($P<0.05$)	252 ($P<0.05$)
Friess *et al.*[26]	1995	MC, DB	247	—	0	247 ($P<0.05$)	247 ($P<0.05$)
Montorsi *et al.*[27]	1995	MC, DB	218 (18 CP)	218 ($P<0.05$)	—	—	218 ($P<0.05$)
Briceno Delgado *et al.*[29]	1998		34 (5 CP)	34 ($P<0.05$)	—	—	34 ($P<0.05$)
Yeo *et al.*[22]	2000	DB	211	—	189 (n.s.)	22 (n.s.)	211 (n.s.)
Overall (significant *v* non-significant)			1229	252 (including 23 CP) *v* 0	301 *v* 210	337 *v* 129	997 *v* 232

CP, chronic pancreatitis; DB, double blind; MC, multicentre; n.s., not significant; *v*, *versus*

closure rates reached a favourable conclusion, whereas two of three[37,42,43] comparing closure time observed a significantly shorter closure time in the treatment groups (significant results in 125 patients *versus* non-significant results in 31).

Discussion

A recent editorial in the *British Journal of Surgery*[44] stressed the importance of systematic reviews in assessing the effectiveness of treatments. Systematic review aims to provide an unbiased summary of the evidence base to inform a clinical or policy question, to identify gaps in the research and to improve the quality of new research. In the practice of 'evidence-based medicine', systematic reviewing plays a crucial role by providing the key information.

The present initial analysis of the gathered evidence indicates that there is probably a benefit in administering somatostatin or octreotide before operation, although the substantial number of studies in which no benefit could be demonstrated should also be noted. The two most recent large-scale studies (one abstract[30] and one conference proceeding[22]), differed substantially in terms of study design (multicentre *versus* single centre), dosage of octreotide (100 µg every 8 h starting during operation *versus* 250 µg every 8 h starting within 2 h before operation), duration of trial (10 *versus* 7 days), overall pancreatic fistula rate (17·8 *versus* 10·0 per cent) and overall postoperative mortality rate (10·0 *versus* 0·5 per cent). Substantial differences also exist among the remaining identified trials. This has rendered more detailed comparison very difficult. For the prophylactic use of somatostatin or octreotide, the timing of administration appears to be a crucial factor. Present data have shown a distinct difference between administering these drugs before, during or after the operation, which is in keeping with a recent analysis by Berberat *et al.*[8].

In theory, a greater benefit for somatostatin or octreotide should be expected in high-risk patients whose pancreatic parenchyma is essentially normal, because of the strong inhibitory effect of such drugs on pancreatic secretion. This was not observed in this analysis. Paradoxically, for those who had chronic pancreatitis with fibrotic pancreatic tissue and reduced pancreatic exocrine function, the impact of somatostatin or octreotide seemed to be more apparent. It is noteworthy that octreotide has been used to relieve the

Table 4 Therapeutic value of octreotide or somatostatin for treatment of established enterocutaneous pancreatic fistula

Reference	Year	Trial type	Subjects	Treatment	Output reduction	Closure rate	Other
Nubiola-Calonge *et al.*[34]	1987	PRC, blind, cross-over	14 ECF (all > 7 days)	OC: 75–100 µg/8 h Group A: OC → placebo, 2 days each ($n=8$) Group B: placebo → OC, 2 days each ($n=6$)	Group A: from 228 ml/day after OC to 498 ml/day (24 h) ($P=0{\cdot}014$) Group B: from 828 to 247 ml/day (24 h) ($P<0{\cdot}01$) Placebo had no effect	79% (overall) in 2–10 (mean 4·5) days	1 allergic reaction, 1 cholestasis, 2 deaths
Scott *et al.*[35]	1990	PRC	15 ECF, 1 PF	OC: 100 µg/8 h for 12 days	—	44% by day 37, including 4 during treatment (1 by operation, 2 refistulated later)	—
Kusuhara *et al.*[36]	1992	RC	12 ECF	OC: 100 µg/8 h First 5 days: no treatment Second 5 days: OC or placebo Third 5 days: reverse	OC group: from 997(52) to 736(28) g/day ($P<0{\cdot}05$) Placebo had no effect	—	—
Torres *et al.*[37]	1992	MC, PRC, cross-over	33 ECF, 7 PF, all > 150 ml/day	SOM: 250 µg/h for 20 days SOM group: 20 Control: 20 Two groups differed in age and initial output ($P<0{\cdot}05$)	50% reduction: 5·2(0·9) *v* 9·8(2·0) days ($P<0{\cdot}05$) 75% reduction: 7·5(1·2) *v* 13·7(3·0) days ($P<0{\cdot}01$)	85 *v* 80% (n.s.) Closure time: 13·9(1·8) *v* 20·4(3·0) days ($P<0{\cdot}05$)	Complications: 35 *v* 79% ($P<0{\cdot}05$); 2 patients had hyperglycaemia
Scott *et al.*[38]	1993	PRC, DB	17 ECF, 2 PF (all > 7 days)	OC: 100 µg/8 h for 12 days OC group: 11 Control: 8	Similar in the two groups	1 *v* 3 (n.s.)	—
Sitges-Serra *et al.*[39]	1993	PRC, DB	20 ECF (all > 29 days)	OC: 100 µg/8 h for > 12 days Group A: placebo → OC, 2 days each ($n=13$) Group B: reverse ($n=7$)	Group A: from 725 to 151 ml/24 h ($P<0{\cdot}02$) Group B: from 218 ml/24 h after OC to 436 ml/24 h ($P<0{\cdot}05$) Placebo had no effect	78% (overall) by 5·8(2·7) days	Continual treatment changed all high-output fistulas into low ones.; injection pain: 15%; 2 deaths
Gerardo Perez and Bernardo Acosta[40]	1994	RC (*v* 50 historical cases)	12 ECF, 7 PF	OC 50 µg/8 h for 21 days All patients received OC	52% by 24 h, 71% by 72 h ($P<0{\cdot}006$)	68% by 36·4(3·9) days ($P<0{\cdot}01$)	2 adverse effects; 2 deaths
Isenmann *et al.*[41]	1994	MC, RC	25 ECF, 20 PF	SOM 250 µg/h for 14 days SOM group: 25 Control: 20	—	Deduced closure rate: 90 *v* 20% by 30 days Closure time: reduced ($P<0{\cdot}05$)	—
Sancho *et al.*[42]	1995	MC, PRC, DB	26 ECF, 5 PF (all > 50 ml/day and < 8 days)	OC: 100 µg/8 h for 20 days OC group: 14 Control: 17	66(4·3) *v* 68(4·7)% by 24 h (n.s.); 60(4·6) *v* 57(4·3)% by 48 h (n.s.); 62(5·0) *v* 66(4·9)% by 72 h (n.s.)	57 *v* 35% (n.s.) Closure time: 7(3) *v* 12(7) days (n.s.)	Injection pain in 16%; 4 deaths (2 each)
Hernandez-Aranda *et al.*[43]	1996	PRC	85 ECF	OC 100 µg/8 h OC group: 40 Control: 45	—	65 *v* 56% (n.s.) Closure time: 18(11) *v* 27(15) days ($P=0{\cdot}002$)	Mortality: 25 *v* 31% (n.s.)

DB, double blind; ECF, enterocutaneous fistula; MC, multicentre; n.s. not significant; OC, octreotide; PF, pancreatic fistula; (P)RC, (prospective) randomized controlled; SOM, somatostatin; *v*, *versus* severe pain associated with chronic pancreatitis on the grounds that it may lower the intraductal pressure caused by pancreatic secretion[45,46].

As reflected in this review, somatostatin and octreotide seem to have a much more definite benefit in the reduction of postoperative pancreatic fistula output. It is noteworthy that some authors prefer to classify this complication by using other terms, such as 'problematic pancreatic drainage'[24] and 'clinical/biochemical (pancreatic) anastomotic leak'[28]. Clearly, there has been no consensus on the definition of pancreatic fistula. Among the six trials, each involving more than 200 patients, some authors have regarded it as drainage with amylase and lipase content more than three times the maximum normal value, exceeding 10 ml per day on or after postoperative day 3[27], or for at least 4 days from day 4 after operation[10,20,26], whereas others define it as drainage greater than 50 ml of amylase-rich fluid on or after postoperative day 10, or pancreatic anastomotic disruption demonstrated radiographically[22]. Fourtanier *et al.*[30] defined pancreatic fistula either biologically, as drainage fluid containing at least four times the normal serum values of amylase for 3 days irrespectively of the amount of output, or as anastomotic leaks demonstrated by fistulography or radiographic contrast studies.

The most recent study by Yeo *et al.*[22] has demonstrated that, where pancreaticoduodenectomy is performed with a low pancreatic fistula rate, the use of octreotide has no demonstrable effect in preventing fistula and other complications. The same authors have demonstrated, in earlier trials, that the incidence of pancreatic fistula is most strongly associated with factors such as patient throughput and underlying disease[9]. Notably, these same authors have demonstrated that, in experienced units, the operation of pancreaticoduodenectomy can be carried out with a low rate of postoperative pancreatic fistula (in the region of 10 per cent) without the use of octreotide[22].

According to the present analysis, somatostatin or octreotide may have a limited role in the treatment of established enterocutaneous pancreatic fistula, in particular for lowering the volume of drainage and the reduction of closure time. On the other hand, even with the favourable result deduced by Isenmann *et al.*[41], the likelihood that either of these drugs can improve the closure rate is probably very small. Other factors, such as the underlying disease, and the type and quality of operation, may have a more significant influence on outcome.

As the use of somatostatin or octreotide, in the circumstances discussed in this review, does not treat the underlying diseases, it is almost certain that such drugs will not influence the mortality rate or survival of transplanted organs. In addition, the adverse effects associated with these drugs, such as the need for continuous infusion, painful injection and gallbladder sludge, should be taken into account. A new analogue of somatostatin, lanreotide, has been developed recently. This new drug appears to have a similar propensity for reducing complications but has not been evaluated within the context of randomized controlled trials[47,48].

The value of systematic reviews and meta-analyses is in bringing together high-quality studies so that weight can be added to individual findings by the pooling of results. For this process to be effective there must be a high degree of concordance between the methodologies used in the studies. In this review, result pooling was rendered difficult by the differences in the regimens used by various investigators. The potential value of an expensive drug that holds out the prospect of reducing postoperative complications is a subject that undoubtedly warrants randomized controlled trials. There is every justification for interested investigators to carry out pilot studies. However, when these studies show that there is the need for further large studies to obtain an answer, it is the responsibility of those most influential in the area concerned to come together and reach agreement on the methodology of large studies.

Overall this review demonstrates that poor quality of previous studies and lack of collaboration between investigators have resulted in there still being no definitive answer to the originally posed questions. For the present, the use of octreotide or somatostatin cannot be recommended for the prevention of postoperative complications of pancreatic surgery outside clinical trials. There is an urgent need for investigators interested in this area to agree to a further multicentre randomized controlled trial with clearly defined criteria on indications, dosage, timing of administration, length of treatment and definition of what constitutes a pancreatic fistula.

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Randomized clinical trial of laparoscopic *versus* open appendicectomy

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Background: Laparoscopy in patients with a clinical suspicion of acute appendicitis has not gained wide acceptance, and its use remains controversial.
Methods: In a randomized controlled trial of laparoscopic *versus* open appendicectomy, 583 of 828 consecutive patients consented to participate. Three hundred and one patients were allocated to open appendicectomy and 282 patients to laparoscopy, 65 of whom required conversion to open appendicectomy. Length of stay in hospital was the primary endpoint, while operating time, postoperative morbidity, duration of convalescence and cosmesis were secondary endpoints.
Results: Intention-to-treat analysis revealed an equally short hospital stay in the two groups (median 2 days). The median time to return to normal activity (7 *versus* 10 days) and work (10 *versus* 16 days) was significantly shorter following laparoscopy. Laparoscopy was associated with fewer wound infections ($P < 0.03$) and improved cosmesis ($P < 0.001$), but the operating time was longer (60 *versus* 40 min). Laparoscopy was associated with more intraperitoneal abscesses (5 *versus* 1 per cent) but, adjusted for a greater number of gangrenous or perforated appendices in this group, the difference failed to reach statistical significance.
Conclusion: Hospital stay was equally short, whereas laparoscopic appendicectomy was associated with fewer wound infections, faster recovery, earlier return to work and improved cosmesis.

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Introduction

Laparoscopic appendicectomy has been shown to be both feasible and safe in randomized comparisons with open appendicectomy. In addition to improved diagnostic accuracy, laparoscopic appendicectomy confers advantages to the patient in terms of fewer wound infections[1–4], less pain[1–9], faster recovery and earlier return to work[2–6,8,9,10–13]. However, laparoscopic appendicectomy is more time consuming[3–6,8,9,11,12,14–16] and is associated with increased hospital costs[13]. It has been argued that the advantages of laparoscopic appendicectomy achieved by experienced laparoscopic surgeons are marginal compared with open appendicectomy, which can also be performed by surgeons in training through a short, cosmetically acceptable incision with minimal complications and a short hospital stay.

Following a calculation of sample size based on the results of a pilot study, a randomized trial was undertaken to compare the outcome of laparoscopic appendicectomy with that of open appendicectomy performed out of hours by surgeons of comparable experience (trainees and senior registrars on duty) designated before randomization. This was based on the hypothesis that laparoscopic appendicectomy would prove superior to open appendicectomy in terms of hospital stay (primary endpoint), with operating time, postoperative morbidity, cosmesis and time to resume normal activity and work as secondary endpoints.

Patients and methods

Over 19 months, 828 consecutive patients with a clinical diagnosis of acute appendicitis, in the absence of contraindications to creation of a carbon dioxide pneumoperitoneum, were included in the study. At one of three participating departments in two university hospitals, patients below 15 years of age were also included. Two hundred and forty-five patients were not randomized (*Table 1*) and underwent a conventional open appendicectomy, but were included in the analysis.

Five hundred and eighty-three patients consented to randomization (*Table 2*). Patients were stratified by age and sex into eight groups: 2–14, 15–44, 45–64 and 65 or more years for each department. Randomization was computer generated using numbered, opaque and sealed envelopes. Following designation of the surgeon, the envelopes were drawn sequentially by the operating room duty nurse when the operating theatre was booked. Patients were well matched for age, sex and weight, but not for severity of appendiceal pathology. Despite randomization, there were significantly fewer patients with gangrenous or perforated appendicitis randomized to open surgery than randomized to laparoscopy or non-randomized patients (*Table 2*). Histological examination was performed on all removed appendices. Registrars or senior registrars on duty performed the operations. A more experienced colleague, primarily a senior registrar, assisted surgeons who had performed fewer than ten laparoscopic operations.

Open appendicectomy was performed through a horizontal muscle-splitting incision in the right iliac fossa. Non-inflamed appendices if encountered were removed in accordance with departmental protocols. The base of the appendix was invaginated at the discretion of the surgeon.

Table 1 Reasons for non-randomization in 245 patients

	No.of patients
Lack of consent	121
Non-adherence to the protocol	30
Suspicion of peritonitis for reasons other than appendicitis	40
Previous abdominal surgery	8
Inability to understand information*	21
Exclusion after entry	2
Medically unfit for pneumoperitoneum	9
Equipment/surgeon not available	6
Unknown	8

*Foreign language, psychiatric disorders

For laparoscopic appendicectomy, pneumoperitoneum to a pressure of 10–12 mmHg carbon dioxide was obtained with a Veress cannula through an incision in or at the umbilicus. A 10-mm laparoscope was introduced and a 5-mm instrumental port was guided visually through the right hypochondrium. If laparoscopy revealed acute appendicitis, a second 10-mm instrumental port was sited in the suprapubic region. The appendicular artery was dissected and divided between haemostatic clips. The appendix was secured at the base with three loop ligatures, divided between the two distal ligatures, and removed through the 10-mm port. The base of the appendix was not invaginated. Laparoscopy was converted to open appendicectomy if technical difficulties, uncertain anatomy or bleeding were encountered. A macroscopically normal appendix was left in place.

In both groups antibiotics were administered according to departmental protocols. If the appendix was gangrenous, a single dose of ampicillin 3 g and metronidazole 1·5 g was administered. In the presence of a perforated appendix the initial dose was followed by ampicillin 1 g and metronidazole 0·5 g three times a day for 3–5 days.

As in comparable trials, the operating time was recorded as the time from the first incision to the placement of the last suture in the skin. The length of hospital stay was recorded as number of days in hospital after the day of surgery.

Patients in both study groups were discharged as soon as possible, i.e. when fully mobilized without need for opiates, and when assistance from a nurse to secure personal hygiene or to dress was no longer required. They were similarly encouraged to resume normal activity and work as soon as they felt fit. Normal activity was defined as return to usual

Table 2 Profile of 828 consecutive patients with acute appendicitis

	Randomized		
	Open appendicectomy ($n = 301$)	Laparoscopic appendicectomy ($n = 282$)	Not randomized ($n = 245$)
Age (years)*	27 (18–40)	26 (18–40)	30 (18–49)
Sex ratio (M : F)	143 : 158	131 : 151	107 : 138
Weight (kg)*	66 (54–80)	65 (56–80)	67 (57–80)
Pathology of appendix†			
Normal	68	53†	43
Phlegmonous	133	99	85
Gangrenous/perforated	100§	130‡	115
Unknown	0	0	2

*Values are median (interquartile range). †All appendices were examined microscopically except those in 50 patients with a non-inflamed appendix that was left in place at laparoscopy. One normal appendix was removed laparoscopically and two normal appendices were removed after conversion to open surgery. ‡$P < 0.05$ *versus* open appendicectomy; §$P < 0.001$ *versus* non-randomized patients (χ^2 test) activity of domestic and social life at the discretion of the patient. For registration of postoperative morbidity, days until return to normal activity (581 patients), work (402 patients) and cosmetic score, all patients were invited to the outpatient clinic 4 weeks after discharge. Eighty per cent of the patients responded.

Wound infection was defined as discharge of pus that required surgical drainage. Intraperitoneal abscess was defined as a fluid collection diagnosed at ultrasonography or computed tomography which contained pus at ultrasonographically guided aspiration. Cosmesis was recorded by the patient on a visual analogue scale (VAS) graded from 0 (excellent) to 10 (poor).

Statistical analysis

A minimum of 186 patients was required in each group for the study to have a power of 90 per cent to detect a reduction of 1 day in hospital stay (primary endpoint) and a power of 80 per cent to detect a reduction of half a week in return to normal activity and work (secondary endpoint) with a probability of type I error of 5 per cent (two-tailed). Statistical tests on other secondary endpoints (operating time, postoperative morbidity and cosmesis) were exploratory, and served to explain or support findings and to suggest further hypotheses for subsequent research. The endpoint data were analysed according to the intention-to-treat principle. Fisher's exact test and χ^2 test were used to compare categorical data, and Mann–Whitney *U* test to compare ordinal data (operating time, time to return to normal activity and work, and VAS score). Because of an anticipated substantial number of non-randomized patients, and uncertainty about the numbers of working patients and follow-up responders, the study period of enrolment of an adequate number of randomized patients was estimated to be 1·5 years.

The study was approved by the local ethics committee of science and performed in accordance with the Helsinki Declaration. Data were recorded on case-record forms and the study was monitored according to good clinical practice.

Results

Of the 282 patients randomized to laparoscopy the procedure was completed successfully in 217 patients, while 65 patients (23 per cent) had the procedure converted to open surgery. Reasons for conversion were mainly difficulties in visualization and dissection of the appendix, but peritonitis, abscess and perforation contributed in 21 patients. In 50 patients laparoscopy revealed a non-inflamed appendix, which was left in place. Of the 301 patients randomized to open appendicectomy, 68 patients had a non-inflamed appendix that was removed (*Table 2*). During the study interval, 83 surgeons operated, equally divided between registrars and senior registrars.

Primary endpoint

The median hospital stay of 2 days was equally short in both groups (*Table 3*).

Secondary endpoints

Postoperative morbidity

Patients randomized to laparoscopy had significantly fewer wound infections but more intraperitoneal abscesses than patients randomized to open appendicectomy (*Table 4*). Of a total of 16 abscesses, 15 occurred in the subgroup of patients with gangrenous or perforated appendicitis. In this subgroup there was no significant difference between laparoscopic and open appendicectomy (*Table 5*). All but one of the pelvic abscesses was treated by ultrasonographically guided percutaneous aspiration ($n = 2$) or drainage ($n = 13$). One patient randomized to open appendicectomy required drainage by open surgery.

There was no injury to the intestine or blood vessels related to the laparoscopic procedure, but there was one caecal leak. Two randomized patients died, one from mesenteric thrombosis (open appendicectomy) and one from acute myocardial infarction in the absence of surgical complication (laparoscopy converted to open surgery).

The outcome in 245 non-randomized patients is presented in *Table 4*.

Operating time

There was a significantly shorter operating time in patients randomized to open appendicectomy compared with laparoscopic appendicectomy (40 *versus* 60 min; *Table 4*).

Table 3 Results of intention-to-treat analysis of hospital stay (primary endpoint) and convalescence (secondary endpoint)

	Open appendicectomy ($n = 301$)	Laparoscopic appendicectomy ($n = 282$)
Hospital stay (days)	2 (2–4)	2 (1–4)
Time to resuming		
Normal activity (days)	10 (7–16)	7 (4–14)*
Work (days)	16 (10–30)	10 (7–20)*

Values are median (interquartile range). $^*P < 0{\cdot}05$ *versus* open appendicectomy (Mann-Whitney *U*-test)

Table 4 Results of intention-to-treat analysis of secondary endpoints

	Randomized		
	Open appendicectomy ($n = 301$)	Laparoscopic appendicectomy ($n = 282$)	Not randomized ($n = 245$)
Operation time*	40 (30–60)	60 (40–75)†	n.a.
Postoperative morbidity			
Wound infection	21	8‡	9
Intraperitoneal abscess	3	13‡	5
Caecal leak	0	1	0
Adhesive ileus	1	2	2
Wound dehiscence	0	0	1
Pneumonia	1	0	0
Cosmesis (VAS)*	2 (1–3)	1 (0–2)§	n.a.
Death	1	1	4

*Values are median (interquartile range). VAS, visual analogue scale; n.a., data not available. †$P < 0·05$, ‡$P < 0·03$, §$P < 0·001$ *versus* open appendicectomy (Mann-Whitney *U*-test, Fisher's exact test)

Table 5 Intraperitoneal abscess in relation to pathology of appendix (intention-to-treat analysis) in randomized patients

	Open appendicectomy		Laparoscopic appendicectomy	
Pathology of appendix	Abscess	No abscess	Abscess	No abscess
Non-inflamed	0	68	1	52
Phlegmonous	0	133	0	99
Gangrenous/perforated	3	97	12 (4*)	118
Total	3	298	13	269

*Converted to open operation

Convalescence

There was a significantly shorter period of convalescence in the laparoscopic group: 7 *versus* 10 days to resumption of normal activity, and 10 *versus* 16 days to resumption of work (*Table 3*).

Cosmesis

Judged on a VAS, both groups scored well, but patients randomized to laparoscopy were more satisfied with the cosmetic result (*Table 4*).

Diagnostic outcome in randomized patients

In 47 of 50 patients with a non-inflamed appendix (34 of 151 women and 13 of 131 men) laparoscopy disclosed conditions that required no further surgical treatment (gynaecological disorders in 24 patients and mesenteric adenitis in six) or failed to reveal any pathology ($n = 17$). Three patients with diverticulitis required conversion to an open operation through an appropriate incision. In the following 4 weeks none of the 50 patients with a macroscopically non-inflamed appendix left in place at laparoscopy developed clinical signs of appendicitis. The false-negative ratio of laparoscopy was 0 (95 per cent confidence interval (c.i.) 0–7 per cent) (none of 50 patients), whereas the false-positive ratio was 1 (95 per cent c.i. 0–3 per cent) (one of 167 patients).

In patients randomized to open surgery, nine of 68 patients with a non-inflamed appendix required surgical treatment of other conditions whereas 59 patients (46 of 158 women and 13 of 143 men) required no further surgery other than removal of a non-inflamed appendix. The positive predictive value of the clinical and laparoscopic diagnosis of acute appendicitis was 77 (95 per cent c.i. 71–81) and 99 (95 per cent c.i. 98–100) respectively.

Discussion

The results of the present study are in keeping with several previous studies. Most studies report a median hospital stay of 2–5 days irrespective of laparoscopic or open surgery[2,4,6,9,12–14,16,17]. Other studies claim a shorter stay following laparoscopy[1,3,5,7,10,11,15]. In none of these studies were either patients or assessor blinded to the operative technique employed, constituting a possible source of bias.

In the present study it is conceivable that the occurrence of more severely inflamed appendices in patients randomized to laparoscopy adversely affected hospital stay. In a recent large series of 4950 patients, perforated appendicitis was associated with a significantly longer hospital stay than non-perforated appendicitis (7 *versus* 3 days)[18].

The operating time, as in ten of 17 other controlled trials, was significantly shorter in patients randomized to open surgery[3–9,12,15,16]. In six trials with relatively small numbers of patients no difference was found[1,2,10,11,13,17]. These studies failed to state the level of surgical experience of the primary operator. By adhering to the principles of intention to treat, the relatively high conversion rate in the present study undoubtedly influenced the operating time. In another study based on a prospective protocol, a similarly high conversion rate was reported, primarily resulting from laparoscopic inexperience[19]. In experienced hands, conversion rates approximating 5 per cent have been claimed[2,5–7,13].

It is difficult to judge the impact of an increased operating time. Patients in whom laparoscopy reveals a non-inflamed appendix benefit from the laparoscopic approach, with avoidance of open surgery as in the present study in a substantial number of patients. In patients randomized to open appendicectomy only nine of 68 patients required surgery for pathology other than appendicitis whereas 59 patients (29 per cent of men and 9 per cent of women) had no further surgery except removal of a non-diseased appendix.

The substantial number of conversions to open surgery in patients randomized to laparoscopy constitutes an interpretational challenge as patients who required conversion to open surgery eventually received the same treatment modality as patients in the comparative group. Analysing data on an intention-to-treat basis could mask possible benefits in 217 patients in whom laparoscopy was completed successfully. From a statistical point of view, however, there is no alternative. Of 17 published randomized controlled studies, no more than four studies adhered to the principle of intention to treat[2,9,10,16]. Furthermore, only four trials stated the level of surgical experience, ensuring the same level in each group[4,12,14,15]. None of the studies provided information as to whether the surgeon was designated before randomization.

In accordance with other studies[1–4,20] there were significantly fewer wound infections in the laparoscopy group. Theoretically, a reduction in wound infection rate can be achieved by extraction of the specimen through a port or with use of an endobag, or leaving a non-inflamed appendix in place. This has been confirmed in the present intention-to-treat analysis on a large number of patients, in spite of the higher proportion of severely inflamed appendices in the group randomized to laparoscopy, which is expected to increase the number of wound infections[18], and the relatively high conversion rate to open surgery.

The prevalence of intraperitoneal abscess formation following open appendicectomy approximates 2–3 per cent[21,22], principally in patients with perforated appendicitis[18,22]. In one retrospective review, laparoscopic appendicectomy was claimed to be associated with a higher rate of intra-abdominal abscess[21]. In another retrospective study that addressed the pathology of the appendix, there was a trend towards a higher rate of abscess formation after laparoscopic removal of perforated appendices, but not for acute phlegmonous appendices[22].

In the present study there were significantly more intraperitoneal abscesses in patients randomized to laparoscopy. There are a number of possible reasons to consider. Although laparoscopy offers the possibility of adequate irrigation and suction of fluid in the peritoneal cavity, it may increase the risk of spreading infected material in the peritoneal cavity. Furthermore, it is possible that the finding is a result of improper laparoscopic technique, for example crushing a gangrenous or perforated appendix instead of grasping its mesentery or failure to clean the peritoneal cavity. This may reflect the level of experience with the procedure among a large number of young surgeons relatively inexperienced with laparoscopy. Most importantly, there were significantly more severely inflamed appendices in patients randomized to laparoscopy. The difference in pathology does not reflect a difference in clinical or operative diagnosis, as the pathology was based on histological examination of all removed appendices. It is therefore most likely that the difference is random.

In the present study the incidence of severely inflamed appendices in non-randomized patients did not differ from that in patients randomized to laparoscopy, nor did the rate of intraperitoneal abscess formation. In addressing this particular complication, six randomized controlled trials[3,8,9,12,14,15], including one intention-to-treat study[9], failed to show any significant increase following laparoscopy.

In the present study all but one abscess were managed by ultrasonographically guided aspiration or drainage, with an uneventful recovery. In a recently published study of appendicectomy in 1024 children, 21 of 23 intraperitoneal abscesses resolved with antibiotic therapy alone[23].

In 11 of 13 controlled trials that have studied postoperative convalescence, convalescence was found to be shorter in patients treated by a laparoscopic approach[2–6,8–13]. In the present study, patients were not blinded to the surgical technique employed, but were equally informed to resume normal activity and work as soon as possible at their discretion. The results show that time to work was reduced from 16 to 10 days by the laparoscopic approach. Less pain in the postoperative period is probably a major contributing factor, as reported in nine of 16 trials[1–9], none in favour of open surgery. Furthermore, one randomized trial indicated that laparoscopic appendicectomy can be cost effective, with a substantial saving of total costs in working patients[13].

Laparoscopic appendicectomy is associated with increased operating time. The general perception is that it has marginal advantages and may not be worth the trouble[24]. However, wound infections are fewer and subjective outcome measures, such as convalescence and cosmesis, are improved. In addition, the diagnostic gain of laparoscopy is indisputable, obviating laparotomy in a substantial number of patients, both men and women. Disorders other than appendicitis that require surgical treatment can be approached through an appropriate incision. Laparoscopic appendicectomy also provides an appropriate procedure for training young surgeons, and may be a safer introduction to laparoscopic skills than laparoscopic cholecystectomy.

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Randomized clinical trial of *Helicobacter pylori* from dental plaque

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Introduction

The isolation of *Helicobacter pylori* from dental plaque has raised the question of whether this is a possible reservoir of infection or reinfection after an apparently successful gastric eradication[1,2]. The aim of this study was to compare the efficacy of systemic drug therapy with that of local periodontal procedures in eradicating *H. pylori* from dental plaque.

Patients and methods

Adult patients were assessed for gastrointestinal problems and recruited if symptoms were sufficient to justify empirical treatment. Patients were excluded if they had symptoms of malignancy, previous investigations for dyspepsia within the past 5 years, were edentulous or had used omeprazole, penicillins, tetracyclines, macrolides, quinolones, nitroimidazoles, bismuth or furazolidone (which are likely to suppress the growth of *H. pylori*) during the previous 6 weeks. A total of 135 patients was recruited, but in 25 no *H. pylori* could be isolated from the plaque and a further 20 either refused treatment or did not attend follow-up appointments. The state of oral hygiene, periodontal and gingival inflammation, denture use and the amount of plaque were recorded[3].

Facilities for detection of *H. pylori* by culture and polymerase chain reaction were not available and therefore smear cytology was used for the diagnosis of *H. pylori*[4].

Six dental plaque specimens were obtained from the lingual surface of the lower incisors and buccal surface of the upper molars. The air-dried smears were stained with Giemsa stain. *H. pylori* was identified when dark bluish-violet-stained, curved, spiral-shaped rods approximately 2–3 μm in length were seen.

Patients with plaque positive for *H. pylori* were randomized to one of the following three treatment regimens. Patients in group 1 received triple therapy (twice-daily omeprazole 20 mg, clarithromycin 500 mg and metronidazole 400 mg). Group 2 received triple therapy plus local dental treatment (dental scaling and chlorhexidine mouth washes twice daily for 7 days). Group 3 received only local dental treatment. Dental plaque specimens were obtained again 10 days after treatment.

Table 1 Summary of results

No. of patients	90
Sex ratio (M : F)	51 : 39
Mean(s.d.) age (years)	41·2(13·4)
Eradication status*	
Group 1	0 of 27†
Group 2	21 of 25‡
Group 3	27 of 30

*Excludes eight patients who refused to comply with treatment. †$P < 0{\cdot}001$ *versus* groups 2 and 3; ‡$P = 0{\cdot}506$ *versus* group 3 (χ^2 test)

Results

Ninety patients were enrolled in the study. Three patients in group 1 and five in group 2 refused to comply with the treatment and were therefore excluded.

Plaque analysis after completion of therapy showed that *H. pylori* was still present in plaque from all the recruits in group 1, but in only four of 25 patients in group 2 and in three of 30 in group 3 (*Table 1*). Eradication rates in groups 2 and 3 were not significantly different.

Discussion

This study shows that effective eradication of *H. pylori* from dental plaque can be achieved by the use of local dental measures. Addition of triple therapy to local dental hygiene does not confer added advantage over local dental hygiene. Thus, it is prudent that a periodontal assessment and treatment aimed at removing dental plaque and improving periodontal health should be added to triple therapy regimens for patients with antral gastritis associated with *H. pylori*[5].

Acknowledgements

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Original article

Treatment of ruptured hepatocellular adenoma

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Background: As the morbidity and mortality rates associated with emergency resection in patients with a ruptured hepatocellular adenoma are high, the authors have favoured initial non-operative management in haemodynamically stable patients.

Methods: A retrospective study was performed to evaluate the treatment of ruptured hepatocellular adenoma.

Results: Over a 21-year interval, 12 patients presented with a ruptured hepatocellular adenoma. Haemodynamic observation and support was the initial management in all 12 patients. Three underwent urgent laparotomy and gauze packing because of haemodynamic instability; no emergency liver resection was necessary. Eight patients had definitive surgery; three developed postoperative complications but none died. Regression of the tumour was observed in three of four patients treated conservatively.

Conclusion: The initial management of a ruptured hepatocellular adenoma should be haemodynamic stabilization. Definitive resection is required for rebleeding or for tumours exceeding 5 cm in diameter. A conservative approach may well be justified in case of regression of an asymptomatic adenoma.

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Introduction

Emergency resection of a ruptured hepatocellular adenoma is associated with high morbidity and mortality rates. While elective resection of a hepatocellular adenoma has a mortality rate of less than 1 per cent, this may increase to 5–10 per cent in those with intra-abdominal haemorrhage[1,2].

In this study the treatment of 12 patients with a spontaneously ruptured hepatocellular adenoma was reviewed.

Patients and methods

This was a retrospective study of 12 patients with a ruptured hepatic adenoma who were managed at University Hospital Rotterdam between 1978 and 1999. Medical records were reviewed with respect to the haemodynamic state on presentation, treatment and outcome. If operation was necessary, a distinction was made between emergency resection within 24 h of admission and (elective) resection after haemodynamic stabilization.

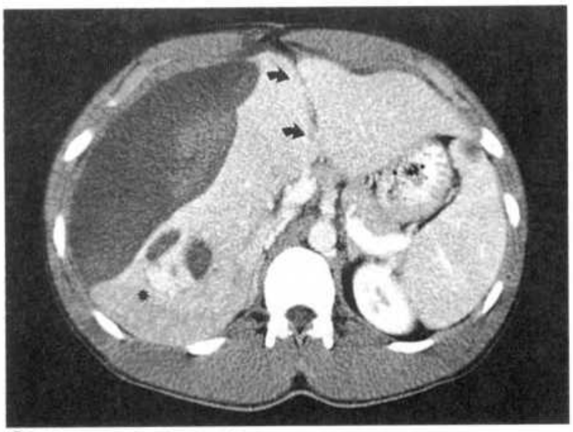
There were ten women and two men, with a mean age of 35 (range 20–49) years. All the women had a history of oral contraceptive use with a mean duration of 10·5 (range 5–20) years. Both men had been using anabolic steroids, for 16 and 4 years respectively. In ten patients bleeding was the first presentation of the hepatocellular adenoma, and two patients were known to have a liver lesion.

Results

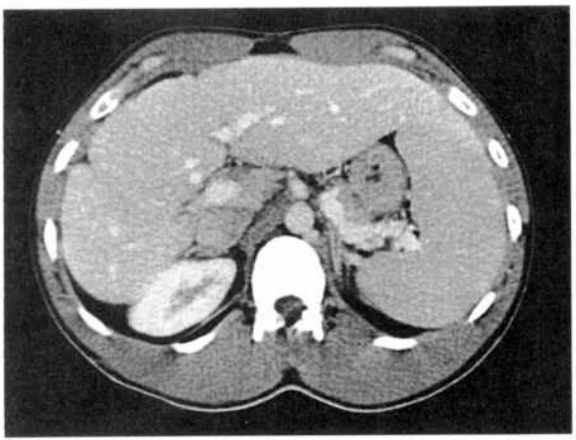
Five patients were in hypovolaemic shock on admission; the remainder were stable. Bleeding was intra-abdominal in five patients, subcapsular in four and intrahepatic in three.

Laboratory tests demonstrated normal α-fetoprotein levels; hepatitis B and C serology (hepatitis B surface antigen, anti-hepatitis B core antigen, anti-hepatitis C virus) was negative in all patients.

Initial management included haemodynamic support. Urgent laparotomy and gauze packing was undertaken in three patients who had persistent bleeding. Eight patients had definitive surgery but no emergency resection was necessary.



a Subcapsular haematoma



b Spontaneous regression after 15 months

Fig. 1 **a** Computed tomogram at presentation showing a ruptured hepatocellular adenoma (asterisk) with subcapsular haematoma. The haematoma has displaced the right kidney (not seen in this figure) caudally and the falciform ligament (arrows) to the left. **b** Computed tomogram at 15 months' follow-up of the same patient, treated conservatively. No adenoma was detectable and total regression of the subcapsular haematoma was noted. The right kidney and falciform ligament have been replaced

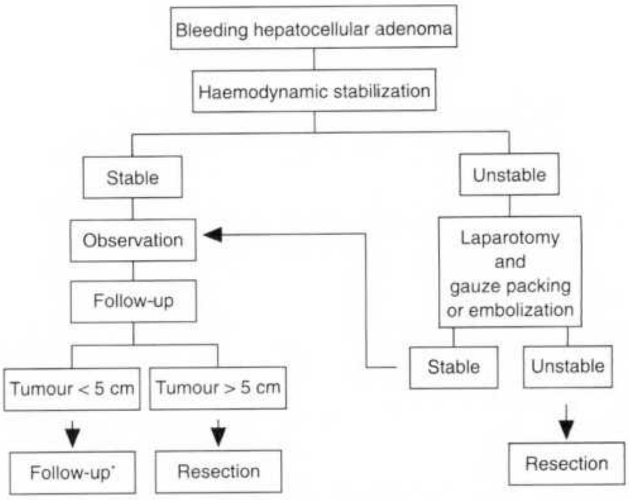


Fig. 2 Algorithm for the management of bleeding hepatocellular adenoma. *Resection is advised in a patient with chronic viral hepatitis

The median interval between presentation and definitive surgery was 10·5 (range 3–64) days. Resection involved right-sided hemihepatectomy (three patients), wedge resection (one) or segmental resection (four). Median intraoperative blood loss was 1950 (range 750–5500) ml. There was no surgery-related mortality. Morbidity included a secondary haemorrhage that necessitated relaparotomy, thrombosis of the femoral and iliac vein, and a wound infection. The median diameter of the resected tumour was 8 (range 4–20) cm; pathological investigation confirmed the diagnosis of hepatocellular adenoma in all patients. Four patients were treated conservatively without resection; two of these were in hypovolaemic shock on initial admission. The duration of follow-up ranged from 7 to 15 months; steroids were not used. Three patients showed regression of the tumour on computed tomography (CT). In two of these patients the tumour was not detectable at the last follow-up (*Fig. 1*). One patient had no sign of regression, and resection was advised.

Discussion

Rupture is the mode of presentation in 50–65 per cent of individuals taking oral contraceptives who have a hepatocellular adenoma[1,3]. Many authors suggest that emergency resection is the preferred treatment for a ruptured hepatocellular adenoma[4,5]. Alternative approaches include hepatic arterial embolization to control bleeding[6]. High mortality rates after emergency resection[1,2] argue for a more conservative approach. Besides, primary non-operative management with or without arterial embolization is currently the preferred treatment for blunt liver injury[7,8]. Although spontaneous bleeding of a hepatocellular adenoma is an intrinsic process, the severity of liver injury may be equivalent to that sustained in major blunt trauma.

For this reason, primary non-operative treatment with haemodynamic stabilization and control of coagulation disorders is recommended. Even the possibility of malignancy is not an argument for acute resection, because intra-abdominal rupture of carcinoma has to be considered as disseminated disease. In case of persistent or recurrent haemorrhage, laparotomy and abdominal packing is an option. In patients treated conservatively, subsequent management could include CT at 3 and 6 months' follow-up, stopping oral contraceptives and avoiding pregnancy (*Fig. 2*). Definitive resection should follow if the tumour diameter exceeds 5 cm after 6 months of follow-up or if rebleeding occurs. This distinction of 5 cm as an indication for resection is in accordance with series described in the literature[6,9], where data suggest that lesion size may be an important indicator of malignant transformation and rupture. If regression is noted, a conservative approach is justified in patients with an asymptomatic adenoma. One exception to these rules is when markers of chronic viral infection (hepatitis B or C) are positive or α-fetoprotein levels are high. In these patients it is virtually impossible for the pathologist to discriminate between adenoma and highly differentiated hepatocellular carcinoma, and resection is advised.

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Original article

Improving operative safety for cirrhotic liver resection

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Background: Liver resection in a patient with cirrhosis carries increased risk. The purposes of this study were to review the results of cirrhotic liver resection in the past decade and to propose safe strategies for cirrhotic liver resection.

Methods: Based on the date of operation, 359 cirrhotic liver resections in 329 patients were divided into two intervals: period 1, from September 1989 to December 1994, and period 2, from January 1995 to December 1999. The patient backgrounds, operative procedures and early postoperative results were compared between the two periods. The factors that influenced surgical morbidity were analysed.

Results: In period 2, patient age was higher and the amounts of blood loss and blood transfused were lower. Although postoperative morbidity rates were similar, blood transfusion requirement, postoperative hospital stay and mortality rate were significantly reduced in period 2. No death occurred in 154 consecutive cirrhotic liver resections in the last 38 months of the study. Prothrombin activity and operative time were independent factors that influenced postoperative morbidity.

Conclusion: With improving perioperative assessment and operative techniques, most complications after cirrhotic liver resection can be treated with a low mortality rate. However, more care should be taken if prothrombin activity is low or there is a long operating time.

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Introduction

Liver resection remains the best therapeutic option for patients with primary or secondary liver malignancies and some benign liver lesions[1–4]. However, liver resection is still a complex procedure associated with high morbidity and mortality rates, especially in patients with cirrhosis[1,5,6]. With improvements in preoperative assessment, anaesthetic technique, operative skill and postoperative care, the risks of liver resection have decreased[6–11]. Several recent studies have reported a postoperative mortality rate of zero after liver resection[9–11]. Management protocols were well described in these reports, but the strategies for management of patients with cirrhosis were not specified.

Taiwan is an endemic area for hepatitis B and so hepatic resection is frequently performed in patients with cirrhosis[12–14]. During the past decade, some management policies regarding cirrhotic liver resection have changed, with extension of patient selection criteria and an increase in operative safety. The aims of this retrospective study were to review the results of cirrhotic liver resection in the past decade and to define safe strategies for this operation.

Patients and methods

Among 781 liver resections performed between September 1989 and December 1999, 359 were performed on 329 patients with cirrhosis (26 patients underwent hepatectomy twice and two patients underwent hepatectomy three times). To examine the changes in outcome of cirrhotic liver resection over time, these operations were studied in two periods according to the date of operation: period 1, from September 1989 to December 1994 (128 operations) and period 2, from January 1995 to December 1999 (231 operations).

Imaging studies (abdominal ultrasonography, computed tomography and angiography), liver function tests (including Child–Pugh grading[15]) and an indocyanine green (ICG) clearance test were carried out before operation. No operation was performed if the ICG 15-min retention rate (ICG R15) was above 50 per cent, the serum total bilirubin level was greater than 68·3 μmol/l (4·0 mg/dl) or prothrombin activity was below 75 per cent of control. Extrahepatic comorbid diseases (heart disease (41 patients), diabetes mellitus (31), lung disease (23), uraemia (four), thalassaemia

Table 1 Extent of hepatectomy in patients with cirrhosis

ICG R15 (per cent)	No. of segments that must be preserved
≥40<50	≥7·5
≥30<40	≥7
≥20<30	≥6
≥10<20	≥5
<10	≥3

Segments were defined according to Couinaud's definition[17]. ICG R15, indocyanine green 15-min retention rate

(one), psychosis (one) and hypothyroidism (one)) were treated before operation to fulfil the criteria of American Society of Anesthesiologists (ASA) grade I or II[16].

According to the ICG R15 value, the extent of hepatectomy was determined by modified Makuuchi's criteria[7–10] and described using Couinaud's definition of liver segmentation[17] (*Table 1*). In period 2, the estimated volume of liver to be preserved at rehepatectomy was one segment or more than the corresponding volume determined by ICG R15 at primary hepatectomy.

Concomitant splenectomy was carried out in patients with hypersplenic thrombocytopenia[18]. Before operation, severe oesophageal varices (presence of red coloured sign or F3[19]) were treated by endoscopic sclerotherapy[1,12]. Incentive spirometry[20] was provided for aged patients (70 years or more) or patients with lung disease. Bowel preparation was performed routinely[21].

Intraoperative central venous pressure, arterial pressure and urine output were monitored, and intraoperative ultrasonography was performed[7–14]. The liver was transected using a Kelly haemostatic clamp[12–14]. Intermittent hepatic inflow blood occlusion, either bilobar or unilobar, was performed during liver transection[7–10,13,14]. A total vascular exclusion technique[22] was carried out for opening major hepatic veins or the inferior vena cava. Blood pressure was maintained using venovenous bypass[23] if necessary. Blood transfusion was restricted to haematocrit below 24 per cent or below 27 per cent in aged patients[15]. Drainage tubes were placed around the cut liver surface which was packed with Surgicel (oxidized cellulose; Johnson and Johnson Medical, Arlington, Texas, USA) after resection.

After operation, intravenous dopamine 5 μg per kg per min was infused continuously for 2–3 days[23]. Cimetidine was prescribed if peptic ulcer was diagnosed. Parenteral solution containing branched-chain amino-acid[24] was used if necessary. Systemic antibiotics were administered routinely in period 1. In period 2, antibiotics were only prescribed in the presence of infective complications[21].

Hospital morbidity and death were defined as complications and deaths that occurred during the same hospital admission. The surviving patients were followed up regularly at outpatient clinics until June 2000.

Patient background and outcome of operations were compared between the two periods. Factors that influenced postoperative morbidity were determined by comparing the preoperative and intraoperative variables of patients with and without complications. Significant variables were selected for multivariate analysis using a forward stepwise logistic regression model.

Continuous variables are presented as median (range) and were compared using the Mann–Whitney *U* test. Frequencies were compared by Pearson's χ^2 test or two-tailed Fisher's exact test. $P < 0{\cdot}05$ was considered significantly different. Long-term survival rates were calculated using the Kaplan–Meier life-table method.

Results

The cause of cirrhosis was posthepatitic in 314 patients, alcoholic in ten, biliary in two and other in three patients. The indication for liver resection was hepatocellular carcinoma in 310 patients, cholangiocarcinoma in five, metastatic colon cancer in five, focal nodular hyperplasia in four, haemangioma in three, pseudotumour in one and hepatic cyst in one patient. The number of operations and frequency of patients with cirrhosis undergoing hepatectomy increased over time (period 1, 128 of 334 hepatectomies; period 2, 231 of 447 hepatectomies; $P < 0{\cdot}0001$). Patient age and the incidence of previous abdominal operations were significantly greater in period 2 than period 1 (*Table 2*). Other background features did not differ significantly between the two periods.

Five operations in period 2 required total vascular exclusion and three operations also required venovenous bypass. One patient with recurrent hepatocellular carcinoma underwent rehepatectomy and removal of tumour thrombi from the right atrium using deep hypothermic circulatory arrest in this period[25].

Details of the extent of liver resection, operative time and blood loss are given in *Table 3*. The volume of operative blood loss and blood transfusion, as well as the number of patients who required blood transfusion, decreased significantly in period 2.

Postoperative complications occurred after 89 operations (30 (23·4 per cent) in period 1 and 59 (25·5 per cent) in period 2; $P = 0{\cdot}70$). Major complications occurred after 86 operations. Some patients had both major and minor complications. The postoperative hospital stay (16 (6–91) *versus* 13 (7–90) days; $P = 0{\cdot}003$) and the mortality rate (six of 128 in period 1 *versus* two of 231 in period 2; $P = 0{\cdot}03$) were

Table 2 Patient profile and preoperative staging of disease

	Period 1 (*n*=128)	Period 2 (*n*=231)	*P*
Sex ratio (M : F)	100 : 28	187 : 44	0·58‡
Age (years)†	56 (22–81)	62 (23–89)	0·04¶
Associated comorbid diseases*	31 (24·2)	76 (32·9)	0·09¶
History of previous abdominal operation*	21 (16·4)	71 (30·7)	0·004‡
Associated oesophageal varices*	24 (18·8)	61 (26·4)	0·89‡
Prothrombin activity (% of control)†	95 (77–100)	95 (74–100)	0·12¶
Platelet count ($\times 10^3$ per mm^3)†	165 (55–378)	154 (28–548)	0·13¶
Serum bilirubin (mg/dl)†	1·2 (0·4–3·2)	1·3 (0·2–3·9)	0·45¶
ICG R15 (%)†	15·4 (3·1–43·1)	17·8 (2·5–44·8)	0·09¶
Child–Pugh grade			0·39§
A	110	189	
B	11	31	
C	7	11	
Tumour size (cm)†	6·8 (1·5–20·0)	6·6 (0·8–26·0)	0·45¶

*Values in parentheses are percentages; †values are median (range); ‡Fisher's exact test; §Pearson's χ^2 test; ¶Mann-Whitney *U* test. ICG R15, indocyanine green 15-min retention rate

Table 3 Intraoperative results of cirrhotic liver resection

	Period 1 (*n*=128)	Period 2 (*n*=231)	*P*
Extent of liver resection**			0·72§
≥3 segments	32	53	
2 segments	38	63	
≤1 segment	58	115	
Concomitant resection	30	49	0·69‡
Diaphragm	24	22	
Stomach	4	5	
Colon	3	5	
Spleen	7	10	
Pancreas	1	0	
Main bile duct	2	4	
Portal vein	2	6	
Inferior vena cava	1	8	
Duodenum	0	1	
Adrenal gland	1	1	
Cardiac tumour thrombi	0	1	
Resected liver weight (g)*	332 (10–3000)	318 (10–2720)	0·29¶
Operative time (h)*	5·4 (1·2–11·5)	5·5 (2·3–15·5)	0·75¶
Estimated blood loss (ml)*	1600 (50–7000)	1050 (100–8000)	0·006¶
Volume of blood transfused (ml)*	0 (0–6500)	0 (0–6000)	<0·0001¶
Patients without blood transfusion†	76 (59·4)	189 (81·8)	<0·0001¶

*Values are median (range); †values in parentheses are percentages; ‡Fisher's exact test; §Pearson's χ^2 test; ¶Mann-Whitney *U*-test; **Couinaud's liver segments[17]

significantly reduced in period 2. The causes of death in period 1 were liver failure in three patients, anaesthetic accident in two and sepsis in one patient. The causes of death in period 2 were pneumonia and hypnotics overdose. No hospital deaths occurred among 154 consecutive hepatectomies in 137 patients in the last 38 months of the study.

Causes of late death included cancer recurrence in 156 patients, liver failure in eight and other causes in three patients. All patients with benign disease survived. The 1-,

Table 4 Univariate analysis of factors influencing the complication rate

	With complication (n=89)	Without complication (n=270)	P
Age (years)†	60 (22–89)	59 (26–86)	0·97¶
Sex ratio (M : F)	74 : 15	213 : 57	0·45‡
Associated comorbid disease*	23 (26)	84 (31·1)	0·42‡
History of previous upper abdominal surgery*	23 (26)	69 (25·6)	0·98‡
Child–Pugh grade			0·11§
A	67	232	
B	17	25	
C	5	13	
Associated oesophageal varices*	21 (24)	64 (23·7)	0·99‡
ICG R15 (%)†	16·8 (2·5–36·8)	16·4 (2·5–44·8)	0·81¶
Serum bilirubin (mg/dl)†	1·3 (0·4–3·2)	1·2 (0·2–3·9)	0·82¶
Prothrombin activity (% of control)†	94 (76–100)	96 (82–100)	0·003¶
Platelet count ($\times 10^3$ per mm^3) †	156 (28–422)	159 (28–548)	0·73¶
Liver ischaemic time (min)†	57 (6–204)	47 (8–189)	0·02¶
Operative time (h)†	6·1 (1·2–15·5)	4·9 (1·3–11·8)	<0·0001¶
Extent of liver resection**			0·75§
≥ 3 segments	20	65	
2 segments	26	75	
≤ 1 segment	43	130	
Tumour size (cm)†	6·4 (0·8–22·0)	5·8 (0·8–26·0)	0·39¶
Resected liver weight (g)†	288 (10–3000)	247 (10–2500)	0·16¶
Operative blood loss (ml)†	1800 (100–7500)	1100 (50–8000)	0·001¶
Volume of blood transfused (ml)†	0 (0–6500)	0 (0–6000)	0·006¶
Period of operation			0·70‡
Period 1	30	98	
Period 2	59	172	
Need for concomitant procedure*	27 (30)	52 (19·3)	0·04‡

*Values in parentheses are percentages; †values are median (range); ‡Fisher's exact test; §Pearson's χ^2 test; ¶Mann-Whitney U test; **Couinaud's liver segments[17]. ICG R15, indocyanine green 15-min retention rate

3- and 5-year actuarial survival rates were 83·3, 63·3 and 55·2 per cent respectively.

Patients with postoperative complications had significantly lower prothrombin activity, a higher incidence of concomitant surgical procedures, longer liver ischaemic time, longer operation time, and greater volumes of operative blood loss and blood transfusion (*Table 4*). Using multivariate analysis, prothrombin activity and operative time were independent factors that influenced surgical morbidity: odds ratio 0·94 (95 per cent confidence interval 0·87–0·96) (P = 0·001) and 1·29 (1·23–1·48) (P = 0·004) respectively.

Discussion

Resection of cirrhotic liver remains challenging, especially in the presence of portal hypertension[1,5,12], because hepatectomy increases portal venous pressure[5,23]. The mortality rate reported after cirrhotic liver resection ranges from 7 to 30 per cent[1,5,6,24]. The common fatal complications in cirrhotic liver resection are liver failure, infection and haemorrhage (from the operative field or from variceal rupture)[1,4–8]. However, some authors have reported series of liver resections with no deaths[9–11].

Most patients with cirrhosis in this study did not undergo a major hepatectomy (defined as resection of three or more Couinaud's segments[2,6,11]). Major hepatectomy was only performed in patients with large lesions and a normal ICG clearance rate. Thus, with circulatory support[23], liver failure resulting from over-resection could be avoided. However, for patients undergoing a rehepatectomy, preservation of functional liver parenchyma and the vascular structure of the remnant liver is more difficult, especially in patients with a deeply seated tumour. This is because the regenerative ability of cirrhotic liver after first hepatectomy is not uniform and the outward appearance of regenerated liver varies[5]. After one death due to resection of too much functioning liver parenchyma in July 1994, stricter criteria for rehepatectomy cases were proposed, with no further deaths occurring after that time.

In period 1, other than hospital deaths, one patient died 3 months after operation from a side-effect of antibiotics

Table 5 Proposal for safe cirrhotic liver resection

- Patient selection criteria
  - Treatable comorbid diseases
  - ASA class I or II
  - ICG R15 < 50 per cent, serum total bilirubin < 4·0 mg/dl and prothrombin activity > 75 per cent of control
  - Liver volume to be preserved fulfills *Table 1* criteria
- Intraoperative strategies
  - Routine use of intraoperative ultrasonography
  - Intermittent hepatic inflow blood occlusion
  - Conservative policy of liver resection (especially in cases of rehepatectomy)
  - Concomitant splenectomy for hypersplenic thrombocytopenia
  - Restrictive policy of blood transfusion

ASA, American Society of Anesthesiologists; ICG R15, indocyanine green 15-min retention rate

(postoperative renal failure). Therefore the strategy of antibiotic usage was changed in period 2. With appropriate antibiotic usage, a high index of suspicion of infective complications and perioperative nutritional support[24], most infective complications can be controlled without side-effects from the antibiotics[21]. However, the incidence of bile leak and intra-abdominal abscess remained high in both periods. Meticulous ligation of small bile ducts[6–11] and use of fibrin glue on the liver cut surface[26] should be performed to prevent these complications.

Under intermittent inflow blood occlusion[7–10,13] and using a restrictive blood transfusion policy[14], the amounts of operative blood loss and blood transfusion were markedly reduced. Owing to the high incidence of associated peptic ulcer in patients with cirrhosis[27], routine preoperative endoscopic examination is essential. Moreover, endoscopy can be used for sclerotherapy for severe oesophageal varices[19], to prevent postoperative gastrointestinal bleeding. With concomitant splenectomy for hypersplenic thrombocytopenia[17], the indications for cirrhotic liver can be extended.

In this series, complication rates did not decrease over time. This may have been because complex procedures (such as resection of portal vein or inferior vena cava, or removal of cardiac tumour) were performed more frequently in period 2. The morbidity after cirrhotic liver resection was comparable to that of other reports[1–3,5–11,24]. After reviewing each cause of death, the operative strategy was changed subtly over time. With increasing experience, most of the complications that occurred in period 2 were treated successfully. Thus, the postoperative hospital stay and hospital mortality rate were reduced, and there were no deaths in the last 38 months. A proposal for 'safe' cirrhotic liver resection, which was adopted in the later part of period 2, is summarized in *Table 5*.

Low prothrombin activity and prolonged operative time were independent factors that caused complications. Because prothrombin activity is also considered an indicator of liver function[5,15], it was included in patient selection criteria for cirrhotic liver resection. The finding of prolonged operative time as an independent factor affecting postoperative complication rates was similar to that reported by Fan *et al.*[11]. Further investigation is needed to better understand how to avoid complications in patients with cirrhosis whose liver resection requires a long operative time.

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Original article

Activation peptide of carboxypeptidase B and anionic trypsinogen as early predictors of the severity of acute pancreatitis

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Background: Early prediction of severity is important in the management of patients with acute pancreatitis. The presence of activation peptides and certain pancreatic proenzymes in plasma and urine has been shown to correlate with severity. This study was designed to assess the value of measuring levels of the activation peptide of carboxypeptidase B (CAPAP) and of anionic trypsinogen.

Methods: Concentrations of CAPAP and anionic trypsinogen were measured in the urine and serum in 60 patients with acute pancreatitis. Preset cut-off levels were used to analyse the accuracy of the tests. Severity was classified retrospectively according to the Atlanta classification.

Results: Concentrations of CAPAP in urine and serum and of anionic trypsinogen in urine correlated with the severity of the pancreatitis. CAPAP in urine showed the highest accuracy. The overall accuracy was 90 per cent, with a positive predictive value of 69 per cent and a negative predictive value of 98 per cent.

Conclusion: In this study, measurement of CAPAP in urine was an accurate way to predict the severity of acute pancreatitis, and was superior to assay of anionic trypsinogen in urine and serum. Measurement of CAPAP in urine may be of value in the management of individual patients with pancreatitis and in the selection of patients for therapeutic trials.

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Introduction

Severe acute pancreatitis remains a condition associated with high morbidity and mortality rates. To improve the outcome, interest has focused on early prediction of severity. Severe pancreatitis requires intensive monitoring and massive fluid resuscitation, and there is increasing evidence that early antibiotic treatment may be beneficial[1–5]. Recent data suggest that treatment with anti-inflammatory agents, such as the platelet-activating factor antagonist lexipafant, might be beneficial, but only if introduced early in the course of disease[6–8]. The success of novel therapies depends on early identification of the 12–25 per cent of patients who progress to a severe outcome, characterized by local complications and/or organ dysfunction.

No single biochemical marker or complex scoring system in clinical use at present has been shown to be ideal for early prediction of severity. The activation of trypsinogen, in or around the pancreas, followed by activation of other pancreatic zymogens, occurs early in the course of acute pancreatitis, in proportion to the extent of pancreatic injury[9,10]. Concentrations of the activation peptide of trypsinogen (TAP) in urine[11,12] and of carboxypeptidase B (CAPAP) in urine and serum[13], as well as anionic trypsinogen (AT)[14,15] in urine and serum, have all shown promise as predictors of severity in acute pancreatitis.

The presence of AT, TAP and CAPAP reflects different pathophysiological events in the course of acute pancreatitis. Levels of immunoreactive AT (irAT) reflect the leakage of unactivated proenzymes from injured acinar cells and levels of immunoreactive TAP (irTAP) correlate with the amount of activated trypsinogen. Trypsin is inactivated by local trypsin inhibitors and activation of other pancreatic zymogens, such as procarboxypeptidase B, will take place only if an imbalance between trypsin and trypsin inhibitors occurs[9]. Immunoreactive CAPAP (irCAPAP) is thus a marker of free trypsin activity which represents a more pronounced state of trypsinogen activation. Levels of irCAPAP may thus reflect one more dimension than irTAP in this respect.

The purpose of this study was to evaluate the accuracy of irCAPAP and irAT in urine and serum, using cut-off levels obtained from earlier studies, in predicting the severity of acute pancreatitis.

Patients and methods

Patients

Urine and serum samples from 60 patients, admitted between 1997 and 1999 to the Departments of Surgery in Malmö, Sweden or Southampton, UK with the diagnosis of acute pancreatitis, were analysed. The diagnosis was based on upper abdominal pain with an increase in serum amylase levels of more than three times the upper reference limit and investigational findings supporting the diagnosis of acute pancreatitis or excluding other causes. Forty-seven patients underwent ultrasonography or computed tomography (CT). Five of the patients who had endoscopic retrograde cholangiopancreatography-induced pancreatitis had a quick regression of symptoms and did not undergo any additional examinations. The eight other patients, who did not undergo either ultrasonography or CT, had a quick regression of symptoms and serum amylase level. All had known gallstones or current abuse of alcohol. Patients were included in the study if samples of urine and serum had been obtained within 72 h of the onset of symptoms. The patients do not represent a consecutive series.

The severity of pancreatitis was classified retrospectively as severe or mild according to the Atlanta classification[16]. The attack was severe (12 patients) if the patient developed single or multiple organ failure, or one or more local complications. In the absence of organ failure or local complication, the attack was classified as mild (48 patients).

Urine samples were available from all patients with severe disease. In the group with mild disease, however, the urine sample was missing from one patient. Serum samples in the severe group were available from ten patients for irCAPAP analysis and from eight for irAT analysis. In mild cases, the corresponding figures were 46 and 45 patients.

Handling of samples

After diagnosis of acute pancreatitis, serum and spot urine samples were collected. Blood samples were allowed to clot, centrifuged and the serum was separated off. Urine and serum samples were then frozen and stored at −20°C until analysed. All analyses were performed in Malmö.

Assays

An enzyme-linked immunosorbent assay developed in this laboratory was used to measure irAT[17]. In serum the assay measures both AT and trypsin in complex with α_1-protease inhibitor. In urine, the method is specific for anionic trypsinogen, as trypsin in complex with α_1-protease inhibitor is not excreted in the urine[18].

A radioimmunoassay developed in this laboratory was used to measure irCAPAP (available from Euro-Diagnostica, Malmö, Sweden). When irCAPAP is measured in serum there is a cross-reaction with procarboxypeptidase B (approximately 10 per cent) whereas in urine the method is specific for irCAPAP, as procarboxypeptidase B is not excreted in the urine[13,19].

Statistical analysis

Preset cut-off levels for predicting severe disease were used. The cut-off levels were chosen from the results of two earlier published patient populations that have been pooled[13,18], resulting in a series of 50 patients (41 with mild and nine with severe pancreatitis). Receiver–operator characteristic (ROC) curves of irCAPAP and irAT levels in urine and serum were used to determine the optimal cut-off levels for this study.

The Mann–Whitney *U* test was used to compare the results. Differences were considered significant when $P < 0{\cdot}05$. Box plots were used for presenting the distribution of concentrations. All data analysis was performed using Statview 4.5 for Macintosh software (Abacus Concepts, Berkeley, CA, USA). The ability of the various tests to differentiate between severe and mild disease was evaluated using a ROC curve. The results for each test were plotted and the area under the curve (AUC) was calculated using MedCalc 4.16 for Windows 95 software (MedCalc Software, Mariakerke, Belgium). An AUC of 1 indicates 100 per cent sensitivity and specificity while an AUC of 0·5 indicates no discriminatory power[20]. Statistical comparison of the different AUCs was done using the same program.

Results

There were 31 women and 29 men; 21 men had mild and eight had severe pancreatitis. The median age was 57 (range 25–89) years in mild and 64 (39–82) years in severe disease. The aetiologies are shown in *Table 1*.

Local complications developed in seven of the severe cases. These included pancreatic necrosis (five patients) and pancreatic abscesses (two). Single organ failure occurred in four patients and multiple organ failure in five. Five of the 12 patients with severe disease died from pancreatitis.

Table 1 Aetiology of acute pancreatitis in 60 patients

	Mild	Severe	Overall
Gallstones	20 (42)	6 (50)	26 (43)
Alcohol	12 (25)	2 (17)	14 (23)
ERCP	7 (15)	0 (0)	7 (12)
Idiopathic	6 (12)	3 (25)	9 (15)
Hyperlipidaemia	2 (4)	1 (8)	3 (5)
Other	1 (2)	0 (0)	1 (2)

Values in parentheses are percentages. ERCP, endoscopic retrograde cholangiopancreatography

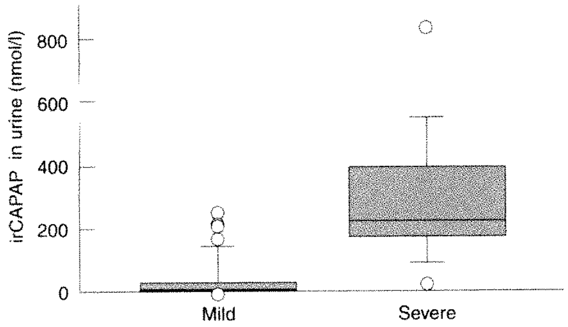

Fig. 1 Immunoreactive carboxypeptidase B activation peptide (irCAPAP) concentration in urine from patients with mild and severe pancreatitis. Median, interquartile range and range are indicated by horizontal bars, shaded boxes and error bars respectively

Immunoreactive carboxypeptidase B activation peptide in urine

Fig. 1 shows the concentrations of irCAPAP in urine according to the severity of the pancreatitis. In severe pancreatitis the median irCAPAP concentration was 229·5 (range 16–840, interquartile range (i.q.r.) 212) nmol/l whereas the corresponding value in mild pancreatitis was 4·5 (range 0–250, i.q.r. 23) nmol/l. This difference was statistically significant ($P < 0·0001$). The AUC, representing the discriminatory power for severe disease, was 0·942 (s.e. 0·048) (*Fig. 2*). Eleven of the 12 patients with severe pancreatitis had an irCAPAP concentration exceeding the preset cut-off level of over 100 nmol/l. In the group of patients with mild disease all but five patients had an irCAPAP concentration below the cut-off level. Sensitivity, specificity and predictive values are shown in *Table 2*.

Immunoreactive carboxypeptidase B activation peptide in serum

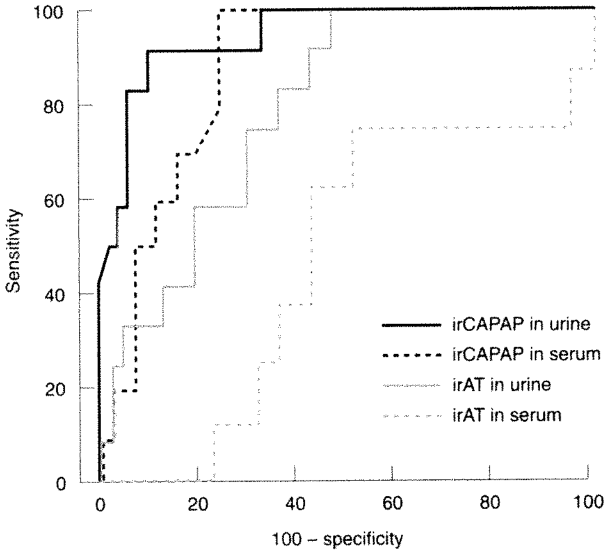

Fig. 2 Receiver–operator characteristic curves showing the accuracy of immunoreactive carboxypeptidase B activation peptide (irCAPAP) and immunoreactive anionic trypsinogen (irAT), in urine and serum, in differentiating between mild and severe acute pancreatitis

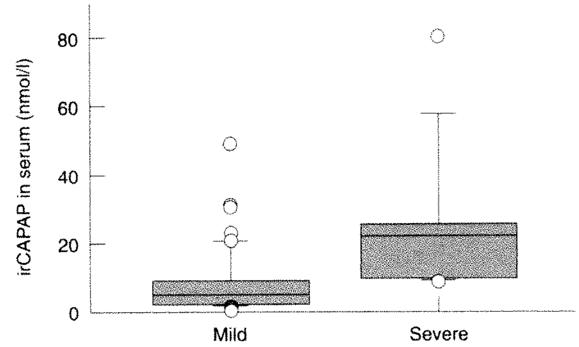

Fig. 3 Immunoreactive carboxypeptidase B activation peptide (irCAPAP) concentration in serum from patients with mild and severe acute pancreatitis. Median, interquartile range and range are indicated by horizontal bars, shaded boxes and error bars respectively

In *Fig. 3* the concentrations of irCAPAP in serum are shown for mild and severe pancreatitis. In severe pancreatitis, the median concentration of irCAPAP in serum was 20·9 (range 8–80, i.q.r. 16) nmol/l compared with 3·1 (range 0–50, i.q.r. 6) nmol/l in mild cases ($P = 0·0002$). The AUC was 0·883 (s.e. 0·072) (*Fig. 2*). All of the ten samples from patients with severe pancreatitis available for analysis had an irCAPAP concentration exceeding the preset cut-off level of over 5·5 nmol/l. In the group of patients with mild pancreatitis, 33 of the 46 had an irCAPAP concentration below the cut-off level. Sensitivity, specificity and predictive values are shown in *Table 2*.

Table 2 Comparison of accuracy in prediction of severity of acute pancreatitis

	Cut-off level	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
irCAPAP in urine (nmol/l)	100	92	89	69	98
irCAPAP in serum (nmol/l)	5·5	100	72	43	100
irAT in urine (µg/l)	3500	58	74	37	88
irAT in serum (µg/l)	1350	38	58	14	84

irCAPAP, immunoreactive carboxypeptidase B activation peptide; irAT, immunoreactive anionic trypsinogen; PPV, positive predictive value; NPV, negative predictive value

Immunoreactive anionic trysinogen in urine

The median concentration of irAT in urine was 4334 (range 430–76 198, i.q.r. 20 490) µg/l in severe pancreatitis and 349 (range 0–40 100, i.q.r. 3436) µg/l in mild pancreatitis ($P = 0{\cdot}0017$). The AUC was 0·796 (s.e. 0·082) (*Fig. 2*). Seven of the 12 patients with severe pancreatitis had an irAT concentration exceeding the preset cut-off level of over 3500 µg/l. Of the 47 patients with mild pancreatitis, 35 had an irAT concentration below the cut-off level. Sensitivity, specificity and predictive values are shown in *Table 2*.

Immunoreactive anionic tryspinogen in serum

The median concentration of irAT in serum was 1269 (range 25–2864, i.q.r. 1015) µg/l in severe pancreatitis and the corresponding value for mild pancreatitis was 1045 (range 87–6746, i.q.r. 1778) µg/l. This difference was not statistically significant ($P = 0{\cdot}8231$). The AUC was 0·475 (s.e. 0·110) (*Fig. 2*). Three of eight patients with severe pancreatitis had an irAT concentration exceeding the preset cut-off level of over 1350 µg/l. Twenty-six of 45 patients with mild pancreatitis had a concentration below the cut-off level. Sensitivity, specificity and predictive values are shown in *Table 2*.

Comparison of receiver–operator characteristic curves (*Fig. 2*)

The significance levels for the differences between the areas under the curves were calculated: irCAPAP in urine *versus* irCAPAP in serum, $P = 0{\cdot}231$; irCAPAP in urine *versus* irAT in urine, $P = 0{\cdot}020$; irCAPAP in urine *versus* irAT in serum, $P < 0{\cdot}001$; irCAPAP in serum *versus* irAT in urine, $P = 0{\cdot}436$; irCAPAP in serum *versus* irAT in serum, $P = 0{\cdot}001$; and IrAT in urine *versus* irAT in serum, $P = 0{\cdot}038$.

Discussion

This study demonstrates that the levels of irCAPAP in urine and serum, as well as those of irAT in urine, obtained within 72 h of the onset of symptoms, correlated well with the severity of an attack of acute pancreatitis.

The differences in the areas under the ROC curves for irCAPAP in urine and serum did not reach statistical significance but, at the preset cut-off levels used in this study, measurement of irCAPAP in urine showed better specificity and predictive values than irCAPAP in serum. The results indicate that irCAPAP in urine may be a better test for predicting the severity of acute pancreatitis. This can partly be explained by the fact that irCAPAP concentrations in urine were almost 10 times higher than those in serum in severe pancreatitis, resulting in a better separation between the mild and severe cases. The results of this and earlier studies indicate that the measurement of an activation peptide is superior to that of leaking proenzymes as a predictor of severe pancreatitis. Since the CAPAP assay cross-reacts, to a certain extent, with procarboxypeptidase B, the presence of procarboxypeptidase B in serum contributes to the immunoreactivity in the circulation. This cross-reaction may be a possible explanation for the less accurate separation between severe and mild pancreatitis in serum samples since some patients with mild disease may have had a high serum concentration of procarboxypeptidase B.

It is noteworthy that eight patients in this study (all with mild disease) had undetectable levels of irCAPAP in urine. This result is in accordance with observations by others[11,21], who have reported that some patients with acute pancreatitis have no detectable irTAP in urine. This suggests that mild acute pancreatitis can occur without detectable trypsinogen activation and activation of other zymogens[22]. It also means that measurement of irCAPAP cannot be used as a diagnostic test for acute pancreatitis, since a substantial proportion of mild cases would not be diagnosed.

Raised levels of irAT in urine and serum have been shown to be accurate tests for the diagnosis of acute pancreatitis, with sensitivities and specificities exceeding 90 per cent[14,23,24]. In prediction of severity, according to the Atlanta classification, irAT in neither urine nor serum reached the high accuracy that was seen when the assays were used for the diagnosis of acute pancreatitis[14]. In the present study, the concentrations of irAT in urine were significantly higher in severe than in mild disease but, at the preset cut-off level, the specificity was only 74 per cent and the sensitivity 58 per cent. The accuracy in predicting severe disease, measured with ROC analysis in a previous study[14], showed similar AUC values to those seen in this study (0·730 *versus* 0·796). In serum, the assay used in this study is not specific for AT since it cross-reacts to some extent with anionic trypsin-α_1-protease inhibitor complexes. With the present assay there was no significant difference in immunoreactivity between severe and mild disease, and the AUC also indicated a lack of discriminatory power.

Generally, the urinary concentrations of both irCAPAP and irAT, in relation to serum levels, were much higher in severe than in mild acute pancreatitis. This may be explained by decreasing renal tubular protein reabsorption in severe pancreatitis. This was shown several years ago during studies on the increased amylase : creatinine clearance ratio in acute pancreatitis[25]. This protein reabsorption insufficiency is probably not specific for pancreatitis but rather reflects a severe state of disease and could account for the differences in urine and serum concentrations in severe and mild pancreatitis.

In conclusion, this study, with preset cut-off levels for estimation of severity, verifies the earlier promising results of the measurement of urinary levels of irCAPAP in the early identification of patients with acute pancreatitis likely to develop severe disease. In this aspect, irCAPAP in urine was more accurate than irAT. Urinary irAT estimation may be valuable for the diagnosis of acute pancreatitis[14,23,24]. In contrast, irCAPAP is not useful for diagnosis of pancreatitis since many mild cases have undetectable levels of the activation peptide. In the future irCAPAP may be useful in the management of individual patients with acute pancreatitis, especially in selecting patients with severe pancreatitis for clinical studies of novel treatments.

The CAPAP test is a radioimmunoassay and, although easy to run (it can be performed in 3 h), is as yet impractical for routine daily clinical work. The development of a quick and simple test is required for clinical application.

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Original article

Procalcitonin strip test in the early detection of severe acute pancreatitis

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Background: Early identification of patients who subsequently develop severe acute pancreatitis would enable the selection of patients who may benefit from early intensive management. Because severe acute pancreatitis is characterized by the development of systemic inflammation the authors studied whether procalcitonin, a marker of systemic inflammation, differentiated between patients with mild and severe acute pancreatitis.

Methods: On admission and 24 h thereafter, serum procalcitonin level was measured by a rapid, semiquantitative PCT®-Q test and serum C-reactive protein (CRP) by an immunoturbidimetric method in a consecutive series of 162 patients with acute pancreatitis. There were 38 severe and 124 mild cases. The accuracy of procalcitonin and CRP in predicting severe acute pancreatitis was compared with that of Ranson and Acute Physiology And Chronic Health Evaluation (APACHE) II scores.

Results: The PCT®-Q test was more accurate in predicting severe acute pancreatitis (sensitivity 92 per cent and specificity 84 per cent at 24 h) than CRP, APACHE II score and Ranson score. Its negative predictive value was high (97 per cent at 24 h), and it detected each patient who developed subsequent organ failure ($n = 22$).

Conclusion: The PCT®-Q test was a useful screening method for detecting severe acute pancreatitis. It is simple and quick to perform and, unlike currently available multiple factor scoring systems, can easily be adopted into routine clinical practice.

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Introduction

Acute pancreatitis is a common disease occurring in 80 per 100 000 inhabitants per year in the USA[1]. It is an acute inflammatory process ranging clinically from mild discomfort with localized inflammation to severe disease involving remote organ systems. There is a continuum from the development of systemic inflammatory response syndrome (SIRS)[2] to the onset of multiple organ dysfunction (MODS), which is seen in about 24 per cent of patients with acute pancreatitis and carries the highest mortality rate of 36 per cent[3,4]. The pathophysiological pathways of necrotizing pancreatitis leading to organ failure are similar to those of other severe inflammatory diseases and conditions such as major trauma and septic shock[5,6].

Most patients with acute pancreatitis recover quickly with simple supportive therapy. There is a pressing clinical requirement to detect patients with the severe form of pancreatitis as early as possible to facilitate immediate treatment in an intensive care unit[7]. Several biochemical parameters[8–11], contrast-enhanced computed tomography (CT)[12,13] and multiple clinicobiochemical scores[14,15] have been used to assess the severity of acute pancreatitis. An ideal prognostic method should be simple, inexpensive, available routinely on a 24-h basis, and possess a high accuracy, especially negative predictive value (NPV), in the early phase of the disease. Such a method is not yet available.

Procalcitonin is a 116-amino acid propeptide of calcitonin with a molecular weight of 13 kDa[16]. Procalcitonin concentration can be determined from either plasma or serum with similar results[17]. It has been introduced as an early marker of severe infection and inflammation[18,19]. Procalcitonin levels have also been reported to increase in endoscopic retrograde cholangiopancreaticography

(ERCP)-induced pancreatitis[20]. Further, procalcitonin has been found in high concentration in infected pancreatic necrosis associated with acute pancreatitis[21].

Recently a rapid semiquantitative test strip, PCT®-Q (BRAHMS Diagnostica, Berlin, Germany), has been developed. This prospective study examined the ability of PCT®-Q test, C-reactive protein (CRP) and multiple factor scoring systems to predict outcome in consecutive patients with acute pancreatitis early in the course of the disease.

Patients and methods

The prospective study population consisted of 162 consecutive patients with less than 72 h of acute symptoms admitted to the emergency unit at Helsinki University Central Hospital between August 1997 and October 1999. There were 55 women and 107 men, with a median age of 47 (range 22–97) years. Acute pancreatitis was diagnosed by characteristic clinical findings (typical epigastric pain, nausea and vomiting) with the presence a raised serum amylase concentration, either more than three times the upper reference limit value alone, or less if associated with the presence of CT findings compatible with acute pancreatitis. The aetiology of acute pancreatitis was alcohol induced in 87 patients, biliary disease in 43, idiopathic in 26 and miscellaneous in six patients. There was no relation between the severity of pancreatitis and sex distribution or aetiology of the pancreatitis (data not shown). CT was performed if the diagnosis of acute pancreatitis was uncertain, if the presence of severe acute pancreatitis was suspected on admission or if the clinical condition deteriorated during admission. CT was performed in 92 patients (57 per cent). Necrotizing pancreatitis was verified by decreased enhancement of the pancreas on CT. The patients were divided into two groups, those with mild acute pancreatitis ($n = 124$) and those with severe acute pancreatitis ($n = 38$), on the basis of the clinical classification of the Atlanta Symposium in 1992[22]. The median interval between the onset of symptoms and admission to hospital was similar in the two groups (24 (range 1–72) h). Five patients (three in the severe group and two in the mild group) were referred from other hospitals.

Twenty-two patients with severe acute pancreatitis developed acute respiratory failure and needed mechanical ventilation. The median duration of mechanical ventilation was 26 (range 1–50) days. Ten of the patients with respiratory failure also needed haemodialysis. The median interval between admission and the onset of organ failure was 2 (range 1–4) days. Seven patients died. The duration of hospital stay was 28 (range 2–140) days in patients with severe acute pancreatitis and 5 (range 1–22) days in those with mild acute pancreatitis.

Blood samples and multiple factor scores

Blood samples were taken on admission to hospital, and 24 and 48 h thereafter. The ethylenediamine tetra-acetic acid-anticoagulated plasma was separated by centrifugation at +4°C and stored at −70°C until use. Serum samples for determination of CRP were collected concurrently. CRP concentrations were determined from the samples taken on admission and 24 h later by an immunoturbidimetric method. The detection limit was 2 mg/l and the reference value less than 10 mg/l. The coefficient of variation was less than 8·5 per cent. Ranson scores were calculated on admission to hospital from the first six admission variables of the score and from variables at 48 h after admission. Acute Physiology And Chronic Health Evaluation (APACHE) II scores were calculated on admission to hospital and 24 h thereafter.

PCT®-Q test

The PCT®-Q tests were done blindly by two trained laboratory assistants unaware of the clinical course of the patients. The test is performed by pipetting 200 µl of the blood sample (plasma or serum) on to the test strip at room temperature and reading the result after 30 min. The PCT®-Q test uses a monoclonal mouse anticatacalcin antibody conjugated with colloidal gold (tracer) and a polyclonal sheep anticalcitonin antibody (solid phase). When the sample (serum or plasma) is applied to the test strip, the tracer binds to the procalcitonin in the sample, forming a labelled antigen–antibody complex. On passing the test band region, the labelled antigen–antibody complex binds to the anticalcitonin antibody immobilized on the solid phase and forms a sandwich complex. At procalcitonin concentrations of more than 0·5 ng/ml this sandwich complex can be seen as a reddish band. The colour intensity of the band is directly proportional to the procalcitonin concentration in the sample. The semiquantitative measurement range of the PCT®-Q test is correlated to three reference concentrations (0·5, 2 and 10 ng/ml). Unbound tracer diffuses into the control band zone, where it is bound by the antitracer antibody and produces an intensely coloured red control band, which is used to check the correct functioning of the test. In the present study, a PCT®-Q test result of less than 0·5 ng/ml was scored as 0, 0·5–2 ng/ml as 1, 2–10 ng/ml as 2, and more than 10 ng/ml as 3. The PCT®-Q test achieves 90–92 per cent of the diagnostic sensitivity and 92–98 per cent of the the specificity of the quantitative procalcitonin assay[23].

Statistical analysis

Comparisons of continuous data were made using the Kruskal–Wallis analysis of variance and Mann–Whitney *U*

Table 1 Ranson and Acute Physiology And Chronic Health Evaluation II scores and serum C-reactive protein concentrations in patients with acute pancreatitis

Variable	Mild ($n = 124$)	Severe ($n = 38$)	P*
Ranson			
On admission	1 (0–4)	2 (0–4)	< 0·01
48 h after admission	0 (0–3)	2 (0–6)	< 0·001
APACHE II			
On admission	4 (0–13)	8 (0–21)	< 0·001
24 h after admission	3 (0–14)	7 (0–22)	< 0·001
CRP (mg/l)			
On admission	15 (5–460)	107 (5–488)	< 0·01
24 h after admission	69 (5–323)	208 (53–475)	< 0·001

Values are median (range). APACHE, Acute Physiology And Chronic Health Evaluation; CRP, C-reactive protein. *Mann–Whitney U test

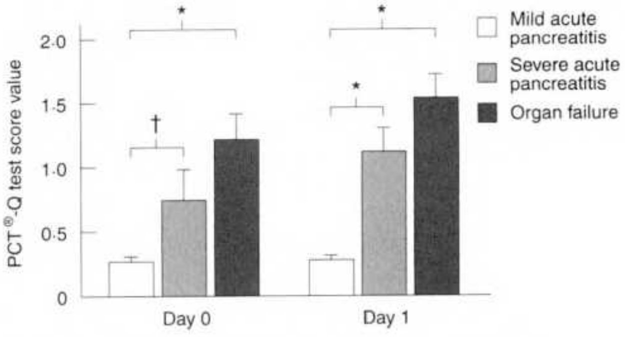

Fig. 1 Mean(s.e.) PCT®-Q test scores in patients with acute pancreatitis on admission to hospital and 24 h later. The scores were 0, 1, 2 and 3 for procalcitonin levels of less than 0·5, 0·5–2, 2–10 and more than 10 ng/ml respectively. $^*P < 0·001$, $^†P < 0·01$ (Mann–Whitney U test)

test, as appropriate. $P < 0·05$ was considered to be statistically significant. Sensitivity (the proportion of patients with severe acute pancreatitis predicted correctly), specificity (the proportion with mild acute pancreatitis predicted correctly), positive predictive value (PPV; true positive rate), NPV (true negative rate) and likelihood ratios were also determined.

Results

Patients with severe acute pancreatitis stayed significantly longer in hospital than those with mild disease ($P < 0·001$). APACHE II and Ranson scores were significantly higher in patients with severe acute pancreatitis than in those with mild disease (*Table 1*). The CRP level was significantly higher in severe acute pancreatitis than in mild disease on admission and 24 h later.

The PCT®-Q test results correlated significantly with the severity of acute pancreatitis on admission and at 24 h (*Fig. 1*; both $P < 0·001$, Kruskal-Wallis test). The sensitivity and specificity of the PCT®-Q test were superior to those of CRP, APACHE II score and Ranson score in predicting severe acute pancreatitis (*Table 2*). At 24 h, the PCT®-Q test had a sensitivity of 92 per cent and specificity of 84 per cent in predicting the development of severe acute pancreatitis. Further, the PCT®-Q test detected patients with subsequent organ failure with a sensitivity of 86 per cent on admission and 95 per cent at 24 h. The sensitivity increased to 100 per cent if the higher of the two procalcitonin levels (admission and 24 h) was used. PPVs were comparable, but the NPVs for the PCT®-Q test were higher than those for APACHE II score and CRP concentration (*Table 2*). Six patients with severe pancreatitis developed a subsequent infective complication; only one was not detected by the dipstick.

Discussion

The PCT®-Q test showed a high sensitivity of 92–95 per cent in predicting severe acute pancreatitis within 24 h of admission and resulted in a very high NPV of 97 per cent at 24 h. After a negative PCT®-Q test severe acute pancreatitis was excluded with a very high probability, which can be considered a preferred feature of a prognostic method. As a consequence, a majority of patients with mild acute pancreatitis can be managed safely in routine wards, which is highly important within limited healthcare resources. The ability of the PCT®-Q test to identify all patients with subsequent organ failure and those with a fatal outcome justifies this conclusion. On the other hand, patients with a positive PCT®-Q test should be referred to specialized clinics experienced in the treatment of patients with severe acute pancreatitis.

Numerous single prognostic markers have been studied for severity grading in acute pancreatitis, but the only marker in routine clinical use is CRP. The main limitation of CRP is the delay of up to 72 h from the onset of symptoms until the peak level of CRP is attained[24,25]. This was also seen in the present study, the sensitivity of CRP on admission being only 37 per cent, which is near that of clinical examination[26]. Recently, a multicentre study reported the accuracy of urinary trypsinogen activation peptide (TAP) as a prognostic marker in acute pancreatitis[27]. TAP showed a high NPV, but a positive TAP result 48 h after the onset of symptoms increased the probability of severe acute pancreatitis only from 20 to 35 per cent[28]. After a positive PCT®-Q test, the likelihood of severe acute

Table 2 Sensitivity, specificity, predictive values and likelihood ratios of PCT®-Q test, C-reactive protein, Acute Physiology And Chronic Health Evaluation II score and Ranson score for discrimination of severe acute pancreatitis

Variable	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	PLR	NLR
PCT-Q						
Day 0	71	84	52	90	4·44	0·35
Day 1	92	84	53	97	5·75	0·10
Day 0 or day 1	95	78	49	98	4·32	0·06
CRP > 150 mg/l						
Day 0	37	88	54	82	3·08	0·72
Day 1	71	68	49	89	2·22	0·43
Day 0 or day 1	74	65	47	89	2·10	0·40
APACHE II ⩾ 8						
Day 0	61	82	51	91	3·39	0·48
Day 1	47	78	40	83	2·14	0·68
Day 0 or day 1	71	73	47	89	2·63	0·40
Ranson ⩾ 3						
Day 0	29	82	33	79	1·61	0·87
Within 48 h	45	98	89	85	22·50	0·56

CRP, C-reactive protein; APACHE, Acute Physiology And Chronic Health Evaluation; PPV, positive predictive value; NPV, negative predictive value; PLR, positive likelihood ratio; NLR, negative likelihood ratio

pancreatitis at all measured time points was more than 65 per cent, and the specificity and NPV of the PCT®-Q test exceeded those of TAP[27]. Further, the PCT®-Q test is easy and quick to perform compared with the laborious enzyme-linked immunosorbent assay for TAP.

The exact site of procalcitonin production in the host is as yet unknown. Procalcitonin is an early marker of severe sepsis[18]. It has been reported to correlate with the severity of infection and to be a valuable marker of the clinical course of patients with sepsis[29–31]. Procalcitonin has also been reported to be an early indicator of the development of severe non-infectious SIRS, to predict pulmonary dysfunction secondary to cardiopulmonary bypass, and to correlate with the severity of SIRS after severe trauma[19,32]. In acute pancreatitis, monitoring circulating procalcitonin levels has been shown to differentiate patients with sterile and infected necrosis[21]. In the present study, one patient with a subsequent infective complication was not detected by the dipstick within the first day of admission. This was probably due to the late development of a pancreatic abscess more than 3 weeks after the hospital admission. Procalcitonin concentration correlates with the severity and predicts the development of organ failure in patients with acute pancreatitis in the very early phase of the disease, which is in accordance with the present results[33].

Ranson and APACHE II scores are commonly used as severity indicators in clinical trials. Ranson criteria include 11 different variables, some of which require a 48-h follow-up to be completed. Results of the present study are in agreement with a recent meta-analysis; the sensitivity of Ranson's signs (29–45 per cent) was lower than that reported for clinical evaluation[26,34]. APACHE II utilizes 12 measurements altogether for physiological variables and additional values for age and chronic health. The accuracy of the APACHE II score with a cut-off of 8 in the present study was superior to that of Ranson's signs in predicting severe acute pancreatitis. However, the APACHE II score could not reach the accuracy of the PCT®-Q test. With a cut-off of 6, the sensitivity of APACHE II score did not improve while the specificity decreased (data not shown). The complexity of multiple factor scoring systems has limited their routine use[35].

A rapid dipstick has not previously been developed for predicting the severity of acute pancreatitis. The PCT®-Q test can be performed easily without laboratory personnel and the result is gained in 30 min. Further, as the present study shows, measurement of this single factor was more sensitive and accurate than the scoring systems in predicting severe acute pancreatitis.

Accurate predictors of severity in the early phase of acute pancreatitis are needed to direct appropriate intensive care unit monitoring and invasive treatment of patients with severe acute pancreatitis as early as possible[7]. Urgent ERCP and common bile duct clearance within 48 h after admission have been reported to be useful for patients with severe biliary acute pancreatitis[36–38]. Further, early enteral feeding and the use of antibiotic prophylaxis are considered beneficial in these patients[39–41]. In the near future, the treatment of severe acute pancreatitis is likely to include means of modifying in a targeted manner the course of systemic inflammation, to prevent the development of MODS[42,43]. A delay of 48 h using Ranson's signs and 72 h using CRP estimation may be detrimental in terms of biological modifier therapy, which should be started with minimum delay[41]. However, immunomodulatory therapies may cause severe side-effects, and patients at low risk of developing MODS should not be exposed[44,45]. The PPV of the PCT®-Q test in the present study was low (on admission to hospital 52 per cent and after 24 h 53 per cent), indicating that additional early markers predicting organ failure and defining the patient's immune inflammatory state are needed before the use of immunomodulatory therapies can be considered.

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Inhibition of matrix metalloproteinases enhances breaking strength of colonic anastomoses in an experimental model

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Background: The breaking strength of colonic anastomoses declines after operation to a minimum at days 3–4, with a subsequent risk of anastomotic dehiscence. The mechanism is thought to be collagen degradation by matrix metalloproteinases (MMPs). This study examined the pathogenic role of MMPs on the mechanical strength of colonic anastomoses by giving the synthetic broad-spectrum MMP inhibitor BB-1101 systemically.

Methods: Forty-eight male Sprague–Dawley rats were treated daily for 7 days with BB-1101 30 mg/kg or vehicle alone (control) starting 2 days before operation. The breaking strength of standardized left-sided colonic anastomoses was measured on postoperative days 1, 3 and 7.

Results: Serum BB-1101 levels were increased at 100 nmol/l in BB-1101-treated rats. The anastomotic breaking strength was 48 per cent higher ($P = 0 \cdot 02$) in BB-1101-treated animals compared with controls on postoperative day 3. Neither collagen accumulation nor infiltration of neutrophils in the anastomotic area was influenced by BB-1101 treatment. Net deposition of new collagen in subcutaneous sponges was unaffected by the BB-1101.

Conclusion: The enhanced breaking strength of colonic anastomoses during the critical early postoperative phase found after administration of a broad-spectrum MMP inhibitor implies that MMPs might increase the risk of anastomotic dehiscence.

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Introduction

Anastomotic dehiscence in the colorectum is associated with high morbidity and mortality rates. In elective surgery, clinically detected leakage is reported to occur in up to 11 per cent of colonic anastomoses and in 12–19 per cent of rectal anastomoses[1–4]. Higher incidences have been reported in the presence of compromising factors, such as low rectal anastomosis and emergency surgery[5].

A decline in anastomotic tensile strength has been found in the early postoperative period in experimental studies[6–8]. During this period the tensile strength of the anastomosis relies on the sutures anchored in the submucosal collagen-rich layer, which constitutes the suture holding capacity. Interstitial collagenases have been assumed to be implicated in the degradation of the existing, mature submucosal collagen, leading to the early impaired breaking strength[9,10]. This is supported by experimental data from Seifert *et al.*[11], who reported maximal collagenolytic activity in colonic anastomoses in rats on postoperative day 3. Furthermore, immunohistochemical and *in situ* hybridization studies have shown that collagenase and other matrix metalloproteinases (MMPs) are in close vicinity to the suture line in uncomplicated anastomotic healing[10,12]. Twenty different MMPs have been identified to date, with different profiles of activity[13]. MMPs are zinc-dependent endopeptidases collectively capable of degrading the components of the extracellular matrix *in vitro*, including fibrillar native collagens. MMPs are upregulated after injury to connective tissues by the inflammatory response[14,15].

MMPs are synthesized as inactive proenzymes, which are activated through cleavage of the propeptide by yet undetermined mechanisms in tissues. *In vitro* MMPs may be activated by other types of proteinases as well as by

MMPs. Neutrophil-derived collagenase (MMP-8), for example, can be activated by matrilysin (MMP-7)[16]. Moreover, Schwartz *et al.*[17] recently demonstrated that neutrophil-derived serine proteinases together with membrane type 1 MMP (MMP-14) activate gelatinase A (MMP-2). These findings may explain the reported beneficial effects of elimination of neutrophils and blocking of serine proteinases on the biomechanical properties of anastomoses[6,18].

Previous attempts to inhibit activated MMPs directly in anastomotic healing have been promising[6,8]. A synthetic thiol metalloproteinase inhibitor transiently reduced the postoperative decline in ileal anastomotic strength in rats[19]. However, these non-specific inhibitors block the activity of all zinc enzymes indiscriminately, with a risk of causing unwanted side-effects[20]. Recently, highly potent synthetic MMP inhibitors of the hydroxamate class suitable for clinical use have been developed and have reached the clinical evaluation phase in oncology.

In the present study, the hypothesis that MMP activity is a major pathogenic factor in the postoperative decline of colonic anastomotic strength was tested by systemic administration of the hydroxamate MMP inhibitor BB-1101 to rats.

Materials and methods

Matrix metalloproteinase inhibitor (BB-1101)

BB-1101 (British Biotech Pharmaceuticals, Oxford, UK) is a synthetic peptide with a molecular weight of 390 Da which chelates the zinc atom at the active site of MMPs. The molecular structure of BB-1101 is published in DiMartino *et al.*[21]. The concentration of BB-1101 required to inhibit by 50 per cent (IC_{50}) the enzymatic activity of MMP-1 (collagenase 1) is 8 nmol/l, that of MMP-2 (gelatinase A) is 4 nmol/l, of MMP-3 (stromelysin 1) 30 nmol/l, of MMP-7 (matrilysin) 30 nmol/l, of MMP-8 (collagenase 2) 3 nmol/l, of MMP-9 (gelatinase B) 3 nmol/l, of MMP-12 (macrophage elastase) 5 nmol/l, of MMP-13 (collagenase 3) 7 nmol/l, and of tumour necrosis α convertase (TACE) 550 nmol/l, as provided by the manufacturer.

Animals and treatments

The study was approved by the local ethics committee of Lund University.

Forty-eight male Sprague–Dawley rats (Möllegaard and Bomullsgaard, Skensved, Denmark) weighing 208–318 g were kept under standard climatic conditions in Makrolon® plastic cages (Scanbur, Lellingegaard, Denmark) with free access to standard pellets (R3, LabFor, Lactamin, Vadstena, Sweden) and tap water. After a 5-day acclimatization period, the animals were divided into three groups of 16, of which eight in each group were allocated pairwise and randomly to treatment with the MMP inhibitor BB-1101 or to treatment with the vehicle only (controls). BB-1101-treated animals and controls were mixed and kept in groups of five.

BB-1101, which is a poorly water-soluble fine powder, was suspended by sonication in the vehicle composed of phosphate-buffered saline (PBS) and ethanol. A dose of 30 mg/kg was injected subcutaneously once daily, in different locations on each occasion. Treatment was started 2 days before operation and continued for 7 consecutive days or until the animal was killed. Control animals were injected with the same volumes of the vehicle alone, following the same injection schedule.

Determination of BB-1101 levels in serum

Before the first injection and at killing, blood samples from the tail vein were collected. The samples were centrifuged and the serum was used for quantitative analysis of BB-1101. The analyses were performed without prior knowledge of the origin of the serum samples using a bioassay with a fluorogenic substrate[22]. Enzymatic cleavage of the substrate releases the highly fluorescent 7-methoxycoumarin group. All activated recombinant MMPs tested to date cleave this substrate, which is inhibited in a dose-dependent manner by synthetic MMP inhibitors. To avoid interference of the assay by endogenous inhibitors such as α_2-macroglobulin, tissue inhibitors of metalloproteinases and other serum proteins, one volume of the serum samples were extracted with six volumes of methanol, which precipitates these proteins. The samples were then centrifuged and the extract removed for assay. Purified human recombinant matrilysin (MMP-7) was activated by aminophenylmercuric acetate 1 mmol/l for 1 h at 37°C and added to the methanol extracts of the serum samples to a final concentration of 200 ng/ml. The enzymatic reaction was started by the addition of the fluorogenic substrate at 6 µmol/l in Tris–hydrochloric acid 50 mmol/l, pH 7·5, sodium chloride 200 mmol/l, calcium chloride 2·5 mmol/l and 0·02 per cent (w/v) Brij-35. The intensity of fluorescence in the samples was quantified by a Fluorstar 96-well plate-reader (BMG Lab Technologies, Aylesbury, UK) with the 320-nm excitation filter and the 405-nm emission filter, expressed as the percentage level of inhibition and compared with a standard curve. The S-shaped semilogarithmic standard inhibition curve was prepared using a stock solution of BB-1101 (10 mmol/l) in dimethylsulphoxide. The solubilized BB-1101 was diluted in PBS and added to serum from uninjured, untreated rats to give a final concentration ranging from 1 nmol/l to

100 μmol/l in threefold steps. The level of inhibition in methanol extracts was measured as described above.

Operations

Surgery was performed under general anaesthesia, induced by subcutaneous injection of a mixture of fentanyl citrate 0·095 mg/kg, fluanisone 3 mg/kg and midazolam 1·5 mg/kg. The operations and breaking strength measurements were performed by the same surgeon, unaware of the treatment given.

Through a midline incision, a 5-mm segment of the left colon 3 cm above the peritoneal reflection was resected and stored at − 70°C until analysed biochemically. A standardized end-to-end anastomosis was then constructed with nine extramucosal, interrupted, single-layer 7/0 polypropylene sutures placed 1·5–2 mm from the wound margin. To standardize the sampling of a 5-mm segment of the anastomotic region at killing, one superficial 7/0 polypropylene suture was placed 2·5 mm proximal to and one suture 2·5 mm distal to the anastomotic line. The laparotomy wound was closed in two separate layers with interrupted 4/0 polybutester sutures, placed 2·5 mm from the wound margin and 5 mm apart.

In the 3 and 7 days study groups, sterile saline-moistened polyvinyl alcohol sponges weighing 60–65 mg (dry weight) and measuring 10 × 10 mm were implanted subcutaneously on the back of the animals to assess hydroxyproline deposition.

The three groups of animals were killed with carbon dioxide on the days 1, 3 and 7 after operation.

Breaking strength measurements of anastomotic and laparotomy wounds

The breaking strength (the maximal force to disrupt the anastomosis) was measured with the sutures in place using a tensiometer with a constantly increasing force of 0·22 N/s. The premarked 5-mm colonic segment with the anastomosis was stored at − 70°C until analysed biochemically. In the animals killed on postoperative day 7 a 10-mm strip from the centre of each laparotomy wound, including skin and underlying abdominal wall, was cut perpendicular to the long axis of the wound with a two-bladed cutting device. The sutures were then removed and the fresh breaking strength was measured with the same tensiometer device and settings.

Biochemical analysis

The 5-mm resected colonic segments were divided longitudinally into equal strips. The strips were analysed for myeloperoxidase (MPO) and hydroxyproline, as indirect measures of neutrophil infiltration and collagen respectively.

Myeloperoxidase

The colonic specimens were weighed and homogenized in 1·5 ml 0·5 per cent hexadecyltrimethylammonium bromide in two cycles, and the homogenates were then centrifuged. The supernatant was freeze–thawed and extracted with chloroform[23]. The solution was centrifuged and the supernatant used for analysis. To 100 μl of supernatant sample, 514 μl of potassium phosphate buffer 0·01 mol/l, pH 6·5, containing 0·0006 per cent hydrogen peroxide and ethylenediamine tetra-acetate 2 mmol/l, was added, followed by the addition of 86 μl *o*-dianisidine dihydrochloride (Sigma, St Louis, Missouri, USA) as hydrogen donor. The absorbency at 460 nm was measured in 15-s intervals, allowing calculation of the reduction of peroxide. One unit of MPO was defined as the amount required to reduce 1 μmol of peroxide per min at 25°C.

Hydroxyproline

The tissue specimens were dried to constant weight. After hydrolysis in hydrochloric acid 6 mol/l for 18 h at 110°C the samples were dried on a hot plate and then washed with distilled water. The drying–washing procedure was repeated three times. The acid-free samples were then dissolved in 2 ml of buffer (1·2 per cent acetic acid, 12 per cent sodium acetate, 5 per cent citric acid, 3·4 per cent sodium hydroxide, pH 6·0). To 1 ml of sample, 0·5 ml of chloramine-T 0·05 mol/l was added, and the samples were incubated for 20 min at room temperature, followed by the addition of 0·5 ml 15·6 per cent perchloric acid and 15 per cent 4-dimethyl aminobenzaldehyde in 1-propanol. After incubation at 60°C for 15 min the absorbency was measured spectrophotometrically at 550 nm and compared with a standard curve for L-hydroxyproline[24].

Statistical analysis

The Mann–Whitney *U* test was used for comparisons between treatment and control groups. $P < 0.05$ was considered statistically significant. Results are given as median (interquartile range (i.q.r.)).

Results

The rats in both groups gained body-weight at a similar rate. In the 7 days study group, the MMP inhibitor-treated

Table 1 Serum levels of the matrix metalloproteinase inhibitor BB-1101 in BB-1101-treated rats and controls

	BB-1101 (ng/ml)		
	Control	BB-1101	*P**
Before treatment	0·0 (0–2)	1·6 (0–4)	0·14
Postop. day			
1	0·0 (0–0)	39·5 (24–48)	< 0·01
3	0·5 (0–2)	53·5 (42–67)	< 0·001
7	0·4 (0–1)	37·3 (28–46)	< 0·001

Values are median (interquartile range). *Mann–Whitney *U* test

rats increased by a median (i.q.r.) of 16 (2–18) per cent and the controls by 18 (16–21) per cent ($P = 0{\cdot}20$). One rat in the day 1 BB-1101 group died from overheating during anaesthesia by a heating lamp. One other rat, in the day 7 BB-1101 group, died during the first postoperative night for unknown reasons. No other complications were observed in any of the groups.

Serum levels of the MMP inhibitor BB-1101 were increased in the BB-1101-treated rats from the first day of sampling (i.e. after 3 days of treatment) and throughout, compared with the controls (*Table 1*). The median (i.q.r.) serum concentration of BB-1101 for all measurements in the BB-1101 group was 43·8 (31·9–53·9) ng/ml, which corresponds to about 100 nmol/l.

Anastomotic breaking strength was low in the early postoperative phase compared with that on day 7, when mechanical strength was regained (*Fig. 1*). However, in the MMP inhibitor-treated animals the anastomotic breaking strength was significantly higher on postoperative day 3 than in controls ($P = 0{\cdot}02$). The median breaking strength was 1·80 (1·53–1·97) N in the MMP inhibitor group on postoperative day 3, compared with 1·22 (0·98–1·68) N in the control animals. The breaking strength of the laparotomy wound, tested on day 7, did not differ significantly between BB-1101-treated animals and controls (12·0 (11·6–13·0) *versus* 12·3 (11·2–12·5) N; $P = 0{\cdot}73$).

The hydroxyproline concentration in the anastomoses, as a measure of collagen, decreased by approximately 40 per cent on postoperative days 1 and 3 compared with values in intact colon at operation. The hydroxyproline concentrations in intact colon at operation and in the anastomotic specimens were similar in the BB-1101 group and controls (*Table 2*). The total content of hydroxyproline in the specimens did not differ between BB-1101-treated animals and controls either (data not shown). The net deposition of new collagens was quantified as the total hydroxyproline content in subcutaneous sponges at postoperative days 3 and 7. No differences between BB-1101-treated animals and controls were noted for either day (*Table 3*).

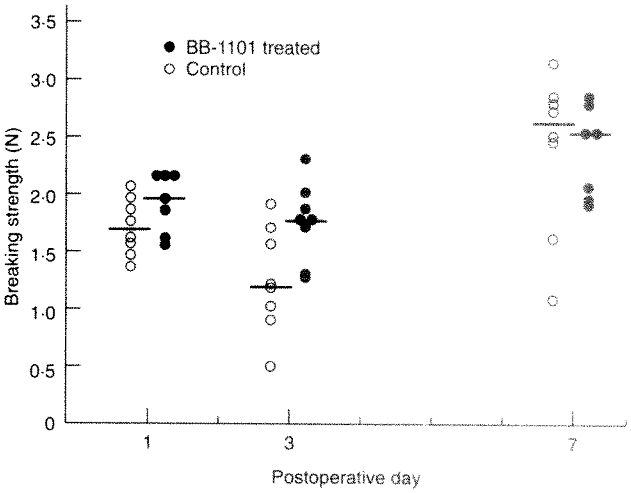

Fig. 1 The matrix metalloprotein inhibitor BB-1101 improved the breaking strength of colonic anastomosis on the critical third postoperative day compared with the value in controls ($P = 0{\cdot}02$, Mann–Whitney *U* test), whereas the difference on postoperative day 1 was not statistically significant ($P = 0{\cdot}10$, Mann–Whitney *U* test). No differences between BB-1101-treated and control animals were found on day 7 ($P = 0{\cdot}82$, Mann–Whitney *U* test). Bars denote median values

Table 2 Hydroxyproline concentration in resected segments of uninjured colon at operation and in colonic anastomotic wounds after operation in BB-1101-treated rats and controls

	Hydroxyproline (µg per mg dry tissue)		
	Control	BB-1101	*P**
At operation	16 (14–17)	15 (12–18)	0·38
Postop. day			
1	9·9 (8·6–12·0)	8·2 (6·6–8·8)	0·083
3	9·8 (6·4–11·0)	9·8 (6·4–11·0)	0·67
7	11·0 (8·0–15·0)	12·0 (9·9–13·0)	0·82

Values are median (interquartile range). *Mann–Whitney *U* test

The neutrophil infiltration in the anastomotic wound was measured indirectly by the specific neutrophilic enzyme MPO. No significant differences between BB-1101-treated animals and controls were noted at any time point, either during or after operation (*Table 4*).

Discussion

The decrease in breaking strength of colonic anastomoses in the early postoperative phase is paralleled by a dramatic decrease in the concentration of existing collagen by up to 40 per cent[7,25]. Interstitial collagenases, which belong to the

Table 3 Net deposition of hydroxyproline in subcutaneous polyvinyl alcohol sponges in the BB-1101-treated and control rats

	Hydroxyproline (μg)		
	Control	BB-1101	P^*
Postop. day			
3	27·5 (21–46)	28·5 (22–42)	0·87
7	876 (749–998)	887 (460–1150)	0·75

Values are median (interquartile range). *Mann–Whitney *U* test

Table 4 Myeloperoxidase activity in resected segments of uninjured colon at operation and of colonic anastomotic wounds after operation in BB-1101-treated and control rats

	Myeloperoxidase (munits per mg wet tissue)		
	Control	BB-1101	P^*
At operation	11 (6–20)	9 (7–14)	0·52
Postop. day			
1	24 (9–42)	11 (6–19)	0·25
3	25 (15–46)	28 (17–38)	0·92
7	15 (13–39)	19 (18–26)	0·42

Values are median (interquartile range). *Mann–Whitney *U* test

MMP enzyme family, have been proposed to cause this loss of mature collagen and hence to contribute to anastomotic dehiscence[9–11]. This study showed a 48 per cent increase in anastomotic breaking strength in animals treated by the broad-spectrum synthetic MMP inhibitor BB-1101 at the time when the anastomosis was weakest.

The loss of existing collagen and mechanical strength in the early stage of anastomotic healing is compensated for gradually by synthesis of new and immature collagen molecules. BB-1101 treatment did not appear to increase the deposition of new collagen, as seen by the similar amounts of hydroxyproline deposited in the subcutaneous sponges at days 3 and 7 in BB-1101-treated animals and controls. The similar anastomotic breaking strength in the BB-1101 and control groups on day 7, when newly deposited collagen contributes significantly to strength, suggests that BB-1101 did not affect the deposition of new collagen in the anastomosis either. Paradoxically, the sum of old and new collagen in the anastomoses, measured as the hydroxyproline concentration, was similar in BB-1101-treated and control animals. However, hydroxyproline measurements are indiscriminate in relation to subtle qualitative properties of collagen such as partial fragmentation, cross-linking and alignment of the collagen molecules[26]. Taken together, these findings suggest that treatment with the MMP inhibitor preserved the existing mature collagen fibrils in the submucosa. Ultrastructural changes of the submucosal collagen in anastomotic healing has been reported[26,27]. An increased breaking strength of intestinal anastomoses without a corresponding increase in collagen concentration has also been reported using other types of proteinase inhibitor[6,8].

The critical role of neutrophils in the early postoperative decline in anastomotic breaking strength and MMP activation is well documented[6,17,18]. Proinflammatory cytokines such as tumour necrosis factor-α (TNF-α) are involved in the recruitment of leucocytes. Hydroxamates, including BB-1101, are capable of inhibiting the release of TNF-α[21,28] and thus, theoretically, the infiltration of neutrophils in the anastomotic area. In the present study no obvious influence of BB-1101 on neutrophil accumulation in the anastomotic wound was noted. This result indicates that the improved breaking strength was not secondary to an impact on the acute inflammatory response, but resulted from a direct blockage of the activity of one or more MMPs. In another study, treatment with the analogous hydroxamate MMP inhibitor GM 6001 attenuated the inflammatory cell infiltration in subcutaneous sponges[28]. However, a higher subcutaneous dose (100 mg/kg) of MMP inhibitor was used[28].

MMPs are required for many processes in wound repair, especially epithelial migration[29,30]. Thus, one concern was whether BB-1101 treatment would impair the healing of the laparotomy wounds. Breaking strength measured at day 7 did not differ between BB-1101-treated animals and controls, suggesting that BB-1101 does not compromise the healing of primary closed skin wounds. Witte *et al.*[28] actually reported enhanced breaking strength of dorsal skin incisions on rats at day 10 with GM 6001 (100 mg/kg). It is, however, likely that non-selective MMP inhibitors delay healing of skin wounds closed primarily by epithelialization.

With the BB-1101 dosage of 30 mg/kg used in the present study, a median serum level of about 100 nmol/l was achieved. The dosage of 30 mg/kg was based on the reports of therapeutic oral dosages of between 10 and 50 mg/kg[21] and almost complete inhibition by BB-1101 100 nmol/l of enzymatic activity in tissue extract of rat glomeruli in substrate gel electrophoresis (zymography)[31]. It is possible that a higher dose of BB-1101 would be therapeutically more efficacious, but with an increased risk of toxicity. Other investigators did not observe any untoward systemic effects when BB-1101 30 mg/kg was used intraperitoneally for 13 days[31], while rats treated with GM 6001 100 mg/kg gained 4 per cent less in body-weight than controls over a 11-day treatment period[28]. In the present study, animals treated with BB-1101 gained body-weight at the same rate as controls. Nevertheless, further optimization of MMP inhibitor therapy is needed with respect to specificity, dosage and route of administration.

In conclusion, treatment with the synthetic MMP inhibitor BB-1101 resulted in an improved breaking strength of colonic anastomosis in the early postoperative period. The mechanism was most likely a direct inhibition of specific MMPs by BB-1101. The results warrant further exploration of synthetic MMP inhibitors in the prevention of anastomotic dehiscence.

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Original article

Tumour necrosis factor inhibitors reduce the acute-phase response in hapten-induced colitis

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Background: Tumour necrosis factor (TNF) α has been implicated in the pathogenesis of inflammatory bowel disease. The aim of this study was to assess the contribution of TNF to the pathogenesis of hapten-induced colitis.

Methods: Colitis was induced in Wistar rats using intracolonic instillation of the hapten trinitrobenzenesulphonic acid (TNBS) in ethanol. Animals were treated with monoclonal anti-TNF antibody (cTN3), an idiotype control antibody (CB0006) or pentoxifylline. Colonic and systemic inflammation was assessed quantitatively.

Results: The use of either TNF inhibitor attenuated the acute-phase response in the early stages of colitis. Median (interquartile range (i.q.r.)) α_2-macroglobulin levels were reduced in animals pretreated with cTN3 (421 (279–915) μmol/ml) or pentoxifylline (567 (253–1454) μmol/ml) compared with levels in untreated colitic animals (1552 (1406–1998) μmol/ml) ($P < 0.001$ and $P = 0.006$, respectively). In established colitis, administration of anti-TNF antibodies resulted in an increase in median (i.q.r.) weight gain (percentage change in body-weight): colitic animals −2·3 (−5·5 to 9·2) per cent *versus* cTN3-treated rats 15 (7·5–16·7) per cent; $P < 0.05$.

Conclusion: The systemic response to TNBS-induced colitis appears to be at least partially dependent on TNF. This study did not provide evidence to support a role for TNF in the pathogenesis of colonic inflammation in this model.

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Introduction

Tumour necrosis factor (TNF) α has been implicated in the pathogenesis of inflammatory bowel disease (IBD). Raised concentrations of TNF have been demonstrated in the faeces of patients with IBD[1] and immunohistochemical methods have demonstrated increased concentrations of TNF protein in intestinal biopsies from patients with active IBD[2]. There is conflicting evidence for an increase in serum TNF concentrations in acute IBD[3,4]. Whilst TNF is undoubtedly present, its role as a primary mediator in IBD is uncertain. Recently studies have demonstrated the effectiveness of single-dose administration of anti-TNF antibodies in the treatment of Crohn's disease[5,6].

The clinical spectrum of IBD is wide. A variety of animal models of intestinal inflammation, both spontaneous and chemically induced, have been used to assess various features of IBD in the laboratory setting[7]. A model of colitis induced by the hapten trinitrobenzenesulphonic acid (TNBS) has been used extensively to investigate the pathogenesis of IBD[8]. The aim of the present study was to investigate the pathogenetic contribution of TNF to colonic and systemic inflammation in the TNBS model of colitis by assessing the effects of TNF inhibitors.

Materials and methods

Two putative therapeutic agents were evaluated in the study: cTN3 (Celltech Ltd, Slough, UK), a chimaeric hamster–mouse antimurine TNF antibody with Fc regions derived from the murine immunoglobulin (Ig) G_1 class[9], and pentoxifylline (PTX) (Hoechst, Hounslow, UK), a xanthine derivative previously used in the treatment of peripheral vascular disease, which is a transcriptional inhibitor of TNF synthesis[10–12]. CB0006, a mouse anti-human IgG_1 that has no anti-TNF activity in the rat, served as an idiotype control for cTN3 to ensure that observed changes were not due to a non-specific IgG effect. The study design is summarized in *Fig. 1*.

All experiments were carried out under the provisions of the UK Animals (Scientific Procedures) Act 1986. Male Wistar rats weighing 250–300 g (Harlan, Shaw's Farm, Oxford, UK) were used in all experiments. There were eight groups of 16 animals in the study designed to evaluate the effects of cTN3, CB0006 and PTX when given either 24 h before or 24 h after the induction of colitis. Animals were housed in groups of four and kept in a 12-h light–dark cycle with free access to tap water and standard laboratory food at all times (SDS diets; Trouw Nutrition, Belfast, UK). Food was kept in hoppers above each cage and weighed twice daily, allowing for calculation of the mean food intake per animal in each group of four. Space limitation precluded individual housing of animals.

Colitis was induced under general anaesthesia using intramuscular ketamine 0·3 ml (Vetalar, ketamine 100 mg/ml; Parke-Davis Veterinary, Pontypoole, UK) and subcutaneous xylazine 0·15 ml (Rompun, xylazine 2 per cent; Bayer UK, Bury St Edmonds, UK). After induction of anaesthesia, a lubricated 8-Ch infant feeding tube (Bard, Crawley, UK) was passed through the anus to a distance of 8 cm (splenic flexure). The inducing agent, 0·6 ml 5 per cent aqueous TNBS (picrylsulphonic acid; Sigma Aldrich, Poole, UK) with 0·25 ml 50 per cent v/v ethanol (TNBS-E), was instilled. Control animals had 0·85 ml saline instilled in the same way. The animals were maintained in a supine Trendelenburg position until recovery from the anaesthetic.

A different dosage schedule was necessary in animals treated with antibodies and in those treated with PTX because the half-life of the antibodies is approximately 7 days[13], whereas PTX has a half-life of 1 h, with active metabolites present for several further hours[14]. The dose of antibody was 15 mg/kg and was derived from results of a preliminary study; it is similar to that used for analogous studies of other antibodies in different forms of colitis[5,15]. The dose of PTX was 20 mg/kg and was based on that used successfully in studies of peritoneal sepsis in rats[16]. In the pretreated group, pentoxifylline injections were begun 24 h before induction of colitis and continued for 72 h. In the post-treatment group, administration began 24 h after induction of colitis and continued for 48 h.

The colitis was allowed to run its course for 8 days after induction at which time the following endpoints were assessed: percentage change in body-weight, colon weight (g), thymus weight (mg), colon macroscopic score (CMS) and serum α_2-macroglobulin concentration (μmol/ml).

The colon weight was determined using the terminal 8 cm of colon opened longitudinally, washed to remove faeces from the lumen, dissected free of adherent small bowel and omentum, and weighed wet on a balance. CMS was assessed by an independent pathologist unaware of the treatment each animal had received, using the scoring system described in *Table 1*[17]. The weight of the thymus was assessed because it had been shown previously to correlate inversely with the severity of colitis in this model[18].

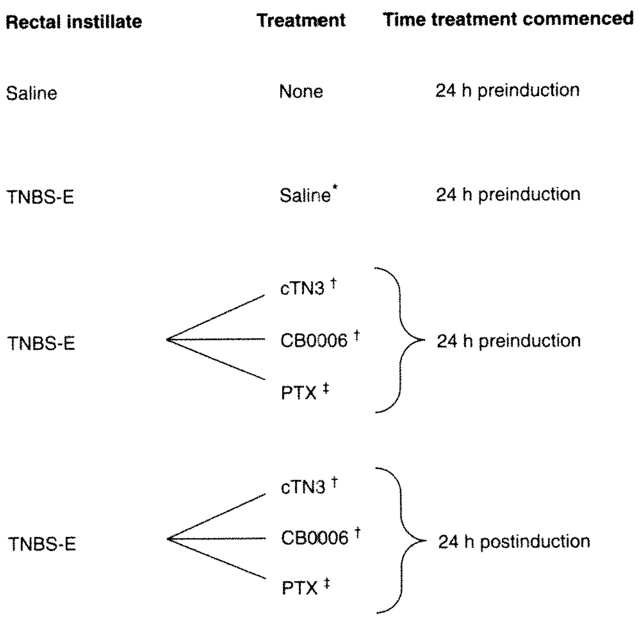

Fig. 1 Study design. *Saline was given as an intraperitoneal injection isovolumetric with the antibody (0·5 ml). †Both antibodies were given as a single intraperitoneal injection of 15 mg/kg. ‡Pentoxifylline (PTX) was given as an intraperitoneal injection of 20 mg/kg at 12-h intervals. TNBS-E, trinitrobenzenesulphonic acid in ethanol; cTN3, monoclonal anti-tumour necrosis factor antibody; CB0006, idiotype control antibody

Table 1 Colon macroscopic score

Score	Criteria
0	No damage
1	Hyperaemia
2	Hyperaemia and thickening without ulceration
3	Ulceration at a single site
4	Two or more sites of inflammation or ulceration
5	Ulceration extending more than 1 cm along the length of the colon
6–10	For damage extending for more than 2 cm along the length of the colon, score increased by 1 for each additional 1 cm of involvement

α_2-Macroglobulin is the major acute-phase protein in rodents and its concentration has been shown to correlate

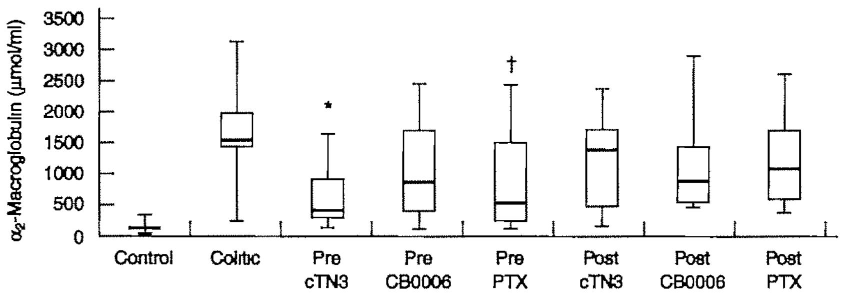

Fig. 2 Serum α_2-macroglobulin concentration 8 days after induction of colitis in rats treated with cTN3, CB0006 and pentoxifylline (PTX) 24 h before or after induction of colitis. Values are median with interquartile ranges. $^*P < 0.001$, $^\dagger P = 0.006$ *versus* untreated colitics (Kruskal–Wallis test)

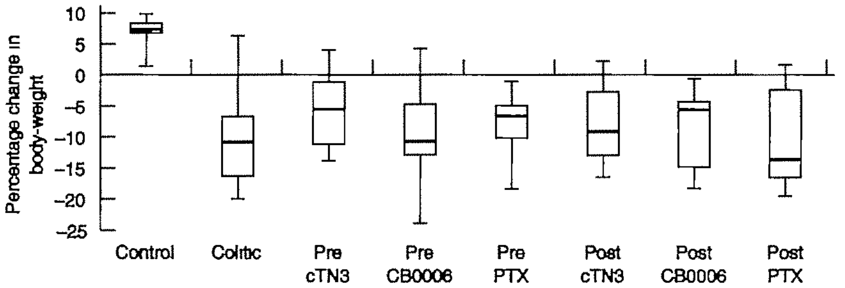

Fig. 3 Percentage change in body-weight 8 days after induction of colitis in rats treated with cTN3, CB0006 and pentoxifylline (PTX) 24 h before or after induction of colitis. Values are median with interquartile ranges

accurately with clinical indicators of inflammation in this model[18]. Blood for assay of α_2-macroglobulin was taken by closed cardiac puncture under general anaesthesia immediately before termination of the experiment. The blood was placed immediately in sterile calcium–ethylenediamine tetra-acetic acid tubes, which were centrifuged at 800*g* for 10 min at 4°C and the supernatant was frozen at −80°C until assay. The concentration of α_2-macroglobulin was determined by a radial immunodiffusion method[19].

In a second experiment the model was allowed to run for 21 days in two groups of eight animals. Colitis was induced as described previously. Seven days after induction of colitis one group of the animals was given a single intraperitoneal dose of cTN3 15 mg/kg. In this study control animals received no treatment. The objective of this experiment was to assess the effect of the cTN3 antibody on established colonic inflammation. All the endpoints described in the initial study were again assessed.

Statistical analysis

In the first experiment differences in means were assessed with the Kruskal–Wallis test. If medians were significantly different, they were compared with the Mann–Whitney *U* test. All variables in the second experiment were compared with the Mann–Whitney *U* test.

Results

In the first experiment preinduction administration of cTN3 reduced the acute-phase protein response (*Fig. 2*) but did not significantly alter loss of body-weight (*Fig. 3*) or measures of colonic inflammation (*Fig. 4*). Administration of PTX before induction of colitis reduced the acute-phase protein response (*Fig. 2*) but did not significantly affect any of the other endpoints. Postinduction administration of cTN3 or PTX had no effect on any measurement of local or systemic inflammation (*Figs 2–4*).

In the second experiment cTN3 had no effect on systemic or colonic inflammation (*Table 2*). However, in the week after treatment experimental animals had more rapid weight gain than controls (*Fig. 5*).

The food intake for each group of four animals was assessed during the study. After induction of colitis there was profound anorexia for 3 days in all animals. By the second week of the study the food intake had returned to preinduction levels and there was no difference between treated and untreated groups. From day 4 until the end of

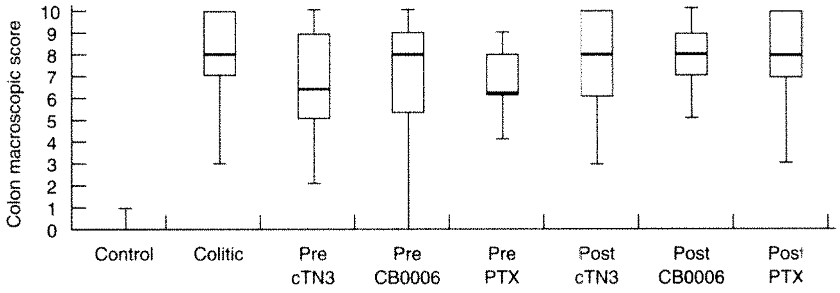

Fig. 4 Colon macroscopic score 8 days after induction of colitis in rats treated with cTN3, CB0006 and pentoxifylline (PTX) 24 h before or after induction of colitis. Values are median with interquartile ranges

Table 2 Results of the 3-week study

Group	CMS	Weight of thymus (g)	α_2-Macroglobulin (μmol/ml)
No treatment	6 (4–10)	327 (169–560)	330 (200–815)
cTN3 at 7 days	4 (4–6)	411 (233–501)	915 (120–1405)

Values are median (interquartile range). CMS, colon macroscopic score; cTN3, monoclonal anti-tumour necrosis factor antibody

the study, treated animals had a median food intake of 17·3 g per day, whereas untreated animals had a median intake of 17·1 g daily. The CMS and serum α_2-macroglobulin concentrations were no different from those in control animals.

Discussion

TNF is a proinflammatory cytokine released from macrophages and T lymphocytes in response to a number of endogenous and exogenous stimuli[20,21]. TNF has a wide variety of effects which include activation of most immune cells and induction of the synthesis of other proinflammatory cytokines, principally interleukin (IL) 1β and IL-6. It is often difficult, however, to correlate increases in serum TNF concentration with these effects[22]. This may be due to early suppression of TNF secretion or to its predominantly paracrine mode of action[23]. As earlier work had demonstrated that serum TNF concentrations were not increased in this model, TNF was not assayed in this study[24].

In this study, pretreatment with cTN3 and PTX attenuated the increase in α_2-macroglobulin concentration seen in the acute phase of the model. The absence of any effect in the CB0006-treated group suggests that the antibody effect was due to an intrinsic effect of cTN3 and not to a non-specific IgG effect. The differences before and after treatment suggest that TNF may have a central role in the initiation of the inflammatory response but that within 24 h of the toxic insult it is of lesser importance. Such a temporal relationship has also been reported in studies of lethal bacteraemia and endotoxaemia where anti-TNF antibodies have attenuated the septic response only when administered before the septic insult[18,25]. The absence of clinical effect on the severity of colonic inflammation by cTN3 and PTX, despite improvements in systemic features in this study, is also interesting. It is known that both ethanol and TNBS can be directly toxic to the colonic epithelium by non-immunological pathways[26] and such injury would not respond readily to immunologically based therapies. The reduction in α_2-macroglobulin concentration produced by cTN3 and PTX was significant. This may suggest a role for TNF in the development of the systemic features of TNBS-induced colitis.

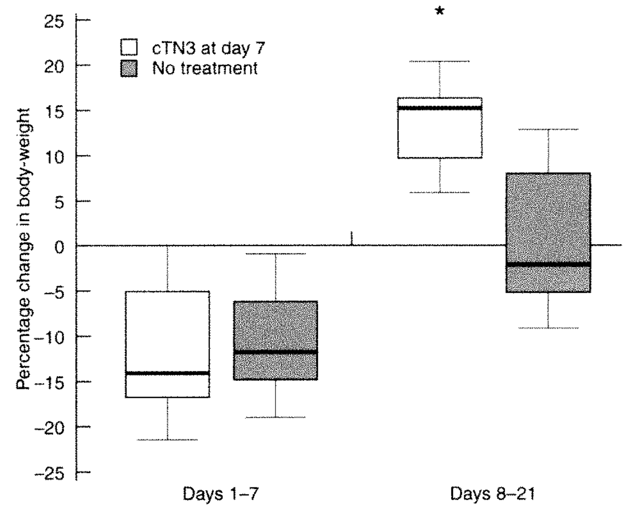

Fig. 5 Effect of cTN3 given 7 days after induction of colitis on percentage change in body-weight. Values are median with interquartile ranges. *$P < 0.05$ (treatment *versus* no treatment, Mann–Whitney *U* test)

It was considered particularly important to use an antibody as a control rather than isonitrogenous albumin for two reasons. First, large infusions of pooled antibody are effective in the treatment of Crohn's disease. Second, antibody controls have not been used in human studies of anti-TNF antibodies to date[27]. The lack of anti-inflammatory effect in the CB0006-treated group is in keeping with work showing that non-specific IgG effects of sufficient magnitude to modify disease in humans can be achieved only after the infusion of several grams of pooled antibody[28], a much greater amount than is necessary to show a clinical effect with anti-TNF antibodies.

The only endpoint in which a significant effect on established disease was demonstrated was with respect to the rate of weight gain after administration of cTN3 in the 3-week study. This may be due to inhibition of the cachectic effects of TNF[29] since the caloric intake of the two groups was not significantly different throughout the study period.

Other groups have investigated the use of anti-TNF antibody treatment in TNBS-induced colitis. Neurath *et al.*[30] demonstrated a reduction in colonic inflammation and a marked reduction in the production of inflammatory cytokines from isolated lamina propria macrophages in mice treated after induction of colitis. Videla *et al.*[31] demonstrated no improvement in colonic inflammation or in the release of eicosanoids from the colon of Sprague–Dawley rats assessed 9 days after induction of colitis. These animals had received an anti-TNF antibody 1 day before and on days 1, 3, 5 and 7 after induction of colitis. Conversely when the antibody was given to animals with established inflammation on days 10, 13 and 16 after induction, and the endpoints were assessed at day 18, eicosanoid release and colonic inflammation were significantly inhibited. Finally, in Wistar rats Neilly *et al.*[32] demonstrated a reduction in circulating α_2-macroglobulin and bioactive IL-6 levels in pretreated animals. The first two studies investigated effects on mice or on different strains of rat from the present work. The results of these studies show some differences from this study. These differences may be a result of the strain and species of animal used, but may also be due to the type of antibody used – its purity, whether it was monoclonal or polyclonal, and whether or not it had any cytotoxic effects.

Another potential difficulty arises from the TNBS model itself. The model results in transmural inflammation and formation of occasional granulomas, bearing a morphological resemblance to clinical IBD[33]. The inflammation is mediated largely immunologically but in the initial 48 h there is considerable direct damage by liberated oxygen radicals[34]. The TNBS model has also demonstrated improvement when animals were treated with established therapies for colitis[35,36]. However, in TNBS colitis, the colitis that develops in captive cotton-top tamarins[37] and in human disease[5,6], there was some reduction in the severity of inflammation either locally in the colon or systemically after treatment with anti-TNF antibodies.

In conclusion, these studies demonstrate that TNF plays an important role in the systemic inflammatory response in the TNBS model of colitis and that this response can be attenuated by use of antibody or pharmacological inhibitors of TNF. It was not possible to demonstrate that TNF mediated the colonic inflammation in TNBS-induced colitis. Similarly, studies with TNF-binding antibodies such as CDP571 have not shown improvements in the macroscopic appearance of colitis in clinical disease. These results suggest that the TNBS model may be suitable for further studies into the mechanisms by which anti-TNF antibodies modulate the systemic inflammatory response in IBD.

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Original article

Transvaginal repair of pouch–vaginal fistula

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Background: The results of surgery for ileoanal pouch–vaginal fistula have been disappointing. Intra-anal procedures result in fistula healing in, at best, approximately 50 per cent of patients. A transvaginal approach was developed to achieve direct access to the internal opening, enabling closure without damage to the anal sphincter.

Methods: Fourteen patients were treated between January 1992 and December 1998. All had had restorative proctocolectomy for ulcerative colitis and all were defunctioned. Histological review confirmed the diagnosis in all patients.

Results: Median age was 40 (range 25–52) years. Median follow-up was 18 (range 6–60) months. The operation was successful in 11 of the 14 patients, after one attempt in six patients, after two attempts in four, and after three attempts in one patient. The operation failed in three patients, who had a permanent ileostomy. The number of bowel actions in the patients who had successful closure ranged from 2 to 10 (median 6) in 24 h, with no faecal incontinence reported.

Conclusion: This simple technique achieved healing in 11 of 14 patients. This compares favourably with the poor results in 28 patients treated by other procedures in the same hospital, with certain healing in five and definite failure in 20.

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Introduction

The reported incidence of pouch–vaginal fistula ranges from 3·6 to 12·0 per cent[1–5] of women undergoing restorative proctocolectomy. Treatment is difficult and surgical closure often fails. A number of surgical approaches have been used. These include peranal, transvaginal and intra-abdominal procedures, but the reported success rate is variable (*Table 1*). *Fig. 1* shows the outcome of the various local repair procedures used at this hospital until 1992. As can be seen, the results were poor. The anal and perineal approaches proved difficult, particularly in gaining access to the internal opening.

Accordingly, a technique has been developed in which the defect is closed transvaginally, allowing a simple approach to the internal opening without compromise to the anal sphincter. This is similar to the technique described for posterior colporrhaphy[6]. Direct exposure of the internal opening through the posterior vaginal wall is followed by closure of the gut tube with resuture of the vagina to create a closed wound.

This technique has been used in all women presenting with pouch–vaginal fistula since 1992 and the results up to the end of 1998 are described.

Patients and methods

Between January 1992 and December 1998, 14 women with pouch–vaginal fistula were operated on via a transvaginal approach (*Table 2*). All patients originally had ulcerative colitis. All colectomy specimens were reviewed histologically and no evidence of Crohn's disease was identified in any patient. The fistula arose from the anastomosis in eight patients and from a site below the anastomosis, but above the anal sphincter, in five. In one patient the fistulous track went through the anal sphincter. Twelve patients had a handsewn and two had a stapled ileoanal anastomosis. The fistula developed a median of 60 days (range from 3 days to 31 months) after operation. Early fistula (within 30 days of operation) developed in five patients.

Complications occurred following the primary ileoanal anastomosis in eight patients, including: postoperative

Table 1 Successful closure of pouch–vaginal fistula in reported series

Reference	Transanal	Fistulectomy	Seton	Transvaginal	Transabdominal	Perineal
Wexner *et al.*[1]	6 of 9	1 of 16		3 of 11	1 of 1	
Groom *et al.*[2]	0 of 3	1 of 3		1 of 1		1 of 5
Keighley and Grobler[3]	1 of 1		4 of 4		1 of 1	
O'Kelly *et al.*[4]				5 of 7		
Lee *et al.*[5]	10 of 20			0 of 1	1 of 1	

Table 2 Clinical details of the 14 patients

	No. of patients
Ulcerative colitis	14
Ileoanal anastomosis	
Handsewn	12
Stapled	2
Fistula origin	
At anastomosis	8
Below anastomosis	6
Time since fistula onset	
< 30 days	5
≥ 30 days	9
Complications	
Pelvic bleed	3
Pelvic sepsis	2
Pyrexia of unknown origin	2
Anastomotic leak	1

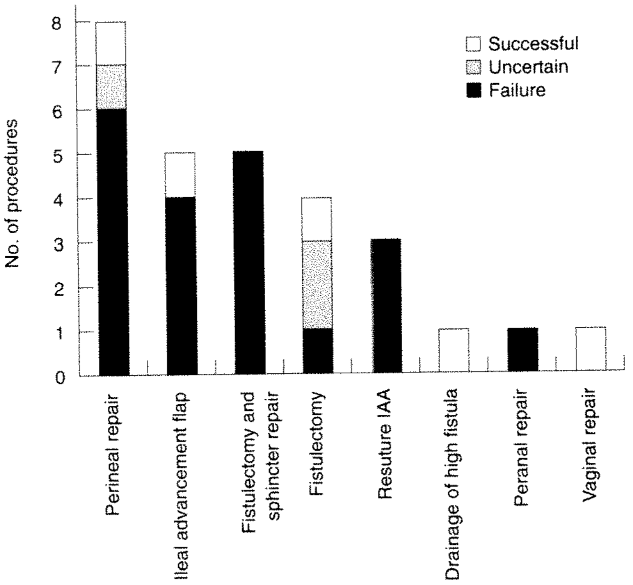
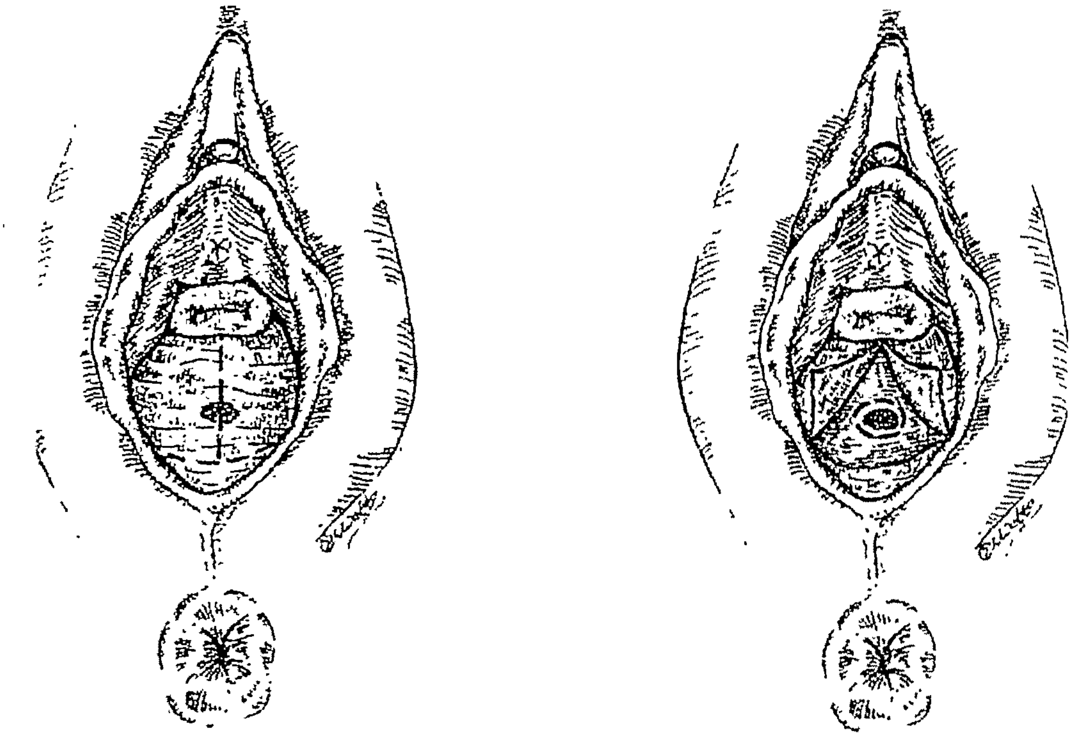


Fig. 1 Results of various techniques for repair of pouch–vaginal fistula in 28 patients treated at St Mark's Hospital between 1975 and 1991. IAA, ileoanal anastomosis

haemorrhage in three, pelvic sepsis in two, unexplained pyrexia in two and an anastomotic leak in one. A median of two examinations under anaesthesia had been performed before attempted fistula closure, usually for drainage of sepsis. In 11 patients the loop ileostomy had not been closed and in three a loop ileostomy was re-formed at the time of fistula surgery. All patients were therefore defunctioned at the time of attempted fistula closure.

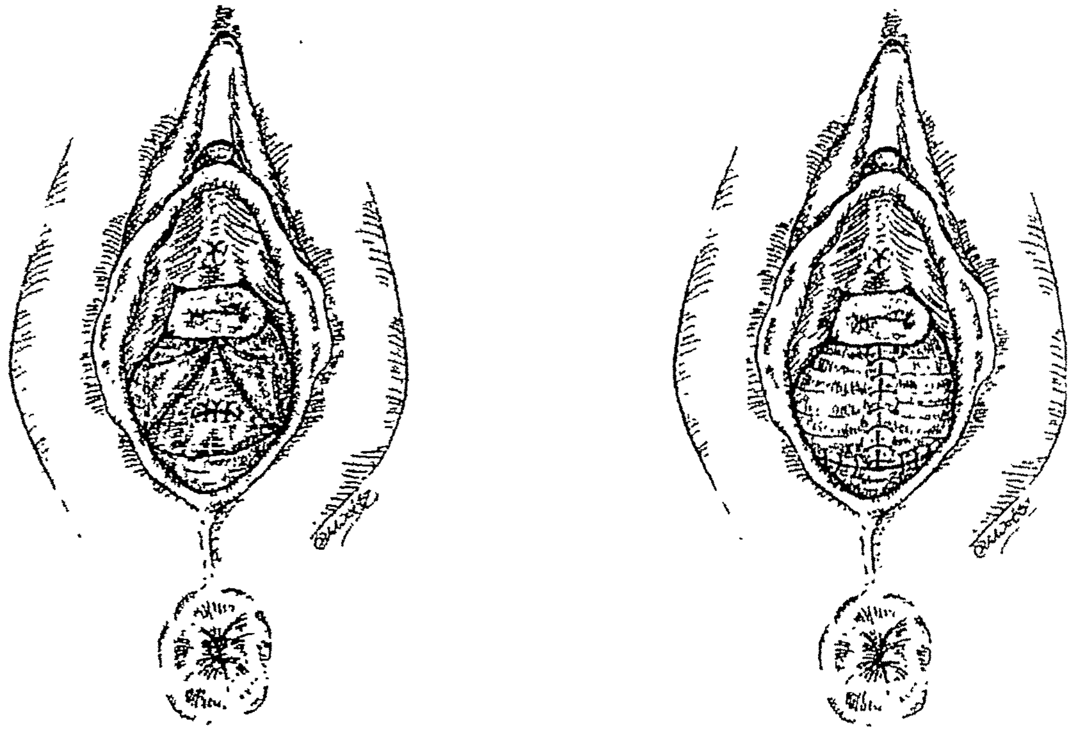
Technique

Mechanical bowel preparation was not performed as the patients were defunctioned after operation. All were given intravenous antibiotics at induction. The patient was placed in the lithotomy position and the bladder was catheterized. An inverted T-shaped incision was made along the midline longitudinal axis of the posterior vaginal wall with the horizontal limb at the junction of the perineal skin with the posterior vaginal wall (*Fig. 2a*). The vagina was dissected from the anal canal and ileal pouch on each side, creating two lateral flaps to expose the anterior wall of the pouch and the ileoanal anastomosis (*Fig. 2b*). The internal opening in the bowel was then excised and the defect closed transversely with interrupted 2/0 polydioxanone sutures (*Fig. 2c*). The vaginal flaps were then replaced and approximated with interrupted 2/0 polydioxanone sutures, leaving a closed wound (*Fig. 2d*). A vaginal pack was inserted to discourage haematoma formation by reduction of the dead space between the vaginal wall and the gut tube. Some 24 h after operation the pack was removed and the patient mobilized. Patients were allowed to eat and drink upon recovery from the anaesthetic, and discharged when fit.



a Incision site

b Lateral flaps raised



c Lateral opening excised and closed

d Flaps replaced

Fig. 2 a–d Technique of transvaginal repair of pouch–vaginal fistula

Results

The median age of the patients was 40 (range 25–52) years. The median follow-up interval was 18 (range 6–60) months. There was no morbidity or death. The operation was successful after one attempt in six patients. Eight patients had a recurrence of the fistula a median of 12 (range 3–20) weeks after operation. Three of these patients declined further repair and had excision of the pouch and formation of a permanent end ileostomy. The remaining five patients had further transvaginal repair with success, after a further one attempt in four patients and a further two attempts in one patient. The loop ileostomy was closed a median of 16 (range 12–40) weeks after successful repair.

The timing of fistulation (early, less than 30 days; late, 30 days or more) had no bearing on the success of fistula repair. Three of five early fistulas, compared with five of nine late fistulas, initially recurred. Subsequently two of three early and three of five late fistulas were repaired successfully.

No stricturing of the anastomosis or pouch dysfunction was observed. The number of bowel actions in the 11 patients who had successful closure ranged from 2 to 10 (median 6) in 24 h, with no faecal incontinence reported. No dyspareunia was noted. There was no death or morbidity as a result of the procedure.

Discussion

Pouch–vaginal fistula is a devastating complication of restorative proctocolectomy and an important cause of failure of the operation. Its aetiology is uncertain. Local tissue ischaemia, Crohn's disease and poor surgical technique are quoted as the possible aetiological basis[7], but postoperative pelvic sepsis is probably the major predisposing factor[2,5]. This is of a similar order to the incidence of anal and perianal disease of 6·7 per cent after restorative proctocolectomy reported in one series[8]. Sepsis is usually due to anastomotic dehiscence, but the evidence from the present study indicates that the fistula may arise from an internal opening in the anal canal, suggesting the possibility of cryptoglandular infection. In this series, eight fistulas arose at the anastomosis and six arose below.

Management has proved difficult. In most series, a proportion of patients are finally diagnosed as having Crohn's disease[3,4] and this usually necessitates pouch excision. However, review of the histological findings in the present series failed to identify any such patient. It has been suggested that late fistula development (more than 30 days after operation) may be due to sepsis resulting from Crohn's disease[2,5] with repair less likely to be successful. In this series, however, the timing of fistulation bore no relation to the ultimate success of repair.

Various surgical procedures have been attempted. Techniques have developed from those used to repair rectovaginal fistulas and are frequently transanal (*Tables 1* and *2*). Ileal advancement flaps, analogous to rectal advancement flaps, have had little success (four failures out of five attempts), but Ozuner *et al.*[7] reported a 50 per cent (ten of 20) closure rate with this technique.

Repair of the fistula via the vagina is much simpler. Access is direct and the anal sphincter is not involved. In 1994 O'Kelly *et al.*[4] demonstrated successful fistula closure in five of seven patients using a vaginal approach. The vagina has an excellent blood supply and the creation of full-thickness flaps is technically straightforward. In addition, further trauma to the anal sphincters is avoided, thus reducing the risk of sphincter injury and subsequent faecal incontinence. Although there is no clear evidence that a defunctioning ileostomy reduces fistula recurrence[5], it is advisable for two reasons: first, because the patient's symptoms need relief; second, to give every chance to the repair.

The small number of patients reflects the rarity of the problem and thus the difficulty of determining optimal surgical treatment. The vaginal approach is technically simple and the results achieved are much better than those obtained with other techniques (*Fig. 1*); it is the preferred technique at this hospital. More advanced techniques such as gracilis[9] or rectus[10] muscle interposition may be used to avoid permanent ileostomy should repeated fistulation occur, although the long-term results of these techniques in larger series are awaited.

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Original article

Sodium hyaluronate enhances colorectal tumour cell metastatic potential *in vitro* and *in vivo*

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Background: Sodium hyaluronate has been used intraperitoneally to prevent postoperative adhesions. However, the effect of sodium hyaluronate on tumour growth and metastasis *in vitro* and *in vivo* is still unknown.

Methods: Human colorectal tumour cell lines SW480, SW620 and SW707 were treated with sodium hyaluronate (10–500 μg/ml) and carboxymethylcellulose (0·125–1 per cent), and tumour cell proliferation and motility were determined *in vitro*. For the *in vivo* experiments male BD IX rats were randomized to a sodium hyaluronate group ($n = 11$; intraperitoneal administration of $0{\cdot}5 \times 10^6$ DHD/K12 tumour cells and 5 ml 0·4 per cent sodium hyaluronate) or a phosphate-buffered saline group ($n = 11$; $0{\cdot}5 \times 10^6$ DHD/K12 tumour cells and 5 ml phosphate-buffered saline intraperitoneally). Four weeks later the intraperitoneal tumour load was visualized directly.

Results: *In vitro* sodium hyaluronate increased tumour cell proliferation and motility significantly. Sodium hyaluronate-induced tumour cell motility appeared to be CD44 receptor dependent, whereas sodium hyaluronate-induced tumour cell proliferation was CD44 receptor independent. *In vivo* there was a significantly higher total tumour nodule count in the peritoneal cavity of the sodium hyaluronate-treated group compared with the control ($P = 0{\cdot}016$).

Conclusion: Sodium hyaluronate enhances tumour metastatic potential *in vitro* and *in vivo*, which suggests that use of sodium hyaluronate to prevent adhesions in colorectal cancer surgery may also potentiate intraperitoneal tumour growth.

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Introduction

Adhesions are defined as abnormal connections between tissues and organs. Considerable morbidity and cost are associated with adhesions and their complications. For adhesions to form there has to be contact between two damaged peritoneal surfaces[1]. The regenerating mesothelium incorporates the two surfaces in the healing process, thus forming an adhesion. Raftery[2,3] described a time period of 5–8 days for adhesions to form in this way. Many adhesion prevention agents have been studied over the years, but attention has concentrated on barrier agents. These work on the basis that, by separating the damaged peritoneal surfaces from one another during the crucial regeneration phase, the incidence of postoperative peritoneal adhesions should be decreased.

In 1995, Burns *et al.*[4] demonstrated in the rat caecal abrasion model that precoating of peritoneal tissues with dilute solutions of hyaluronic acid decreased the incidence of postoperative peritoneal adhesions. Barrier agents incorporating the essential component of sodium hyaluronate with various vectors (e.g. carboxymethylcellulose and phosphate-buffered saline) have been developed. In a multicentre trial Becker *et al.*[5] showed that a sodium hyaluronate-based bioresorbable membrane was efficacious in preventing postoperative peritoneal adhesions when used at operation in patients with ulcerative colitis or familial polyposis. In a similar study Diamond[6] employed a solution of sodium hyaluronate and showed that it also reduced postoperative peritoneal adhesions when used in gynaecological laparotomy. Sodium hyaluronate appears to be safe in operations on patients with benign disease, but its safety has never been investigated with regard to patients with malignant conditions.

CD44 was recognized as a leucocyte antigen in 1982. CD44 proteins participate in molecular processes that involve binding to ligands (e.g. hyaluronic acid, collagen, fibronectin). The CD44 receptor is expressed on a gene on chromosome 11. Alternative splicing of ten different variant exons (v1–v10) on this gene is responsible for the generation of several splice variants of CD44. Some of these splice variants have been found on the surfaces of pancreatic, mammary and colorectal tumour cells, and discovered to correlate with increased metastatic capability of the tumour. Adenomatous polyps from the colon, primary colonic carcinomas and colonic carcinoma metastases all stain very strongly for CD44V, an isoform of CD44 that has been shown to increase the number of metastases when overexpressed in tumour cells in animal models[7,8]. Colorectal tumour cells expressing hyaluronic acid also appear to have increased invasiveness and metastatic potential[9]. It is well established that hyaluronic acid is a major ligand of the CD44 receptor.

Thus sodium hyaluronate functions efficiently to decrease adhesion formation but appears to have the potential to increase the metastatic capability of colorectal tumour cells. The aim of this study was to evaluate the effect of sodium hyaluronate on colorectal tumour cell proliferation and motility *in vitro* and on intraperitoneal tumour growth *in vivo*.

Materials and methods

Human and rat colorectal tumour cell lines

Three human colorectal tumour cell lines, SW480, SW620 and SW707, were obtained from the American Type Culture Collection (Rockville, Maryland, USA). These cells were grown in medium L-15 supplemented with 10 per cent fetal calf serum (FCS), penicillin 100 units/ml, streptomycin sulphate 100 µg/ml and glutamine 2 mmol/l at 37°C in a humidified 5 per cent carbon dioxide atmosphere. Rat colorectal tumour cell line DHD/K12 was obtained from the European Collection of Cell Culture (ECACC) and cultured in Dulbecco's modified Eagle's medium and Ham's F10 medium in a 1 : 1 ratio, supplemented with 10 per cent FCS, penicillin 100 units/ml, streptomycin sulphate 100 µg/ml and glutamine 2 mmol/l at 37°C in a humidified 5 per cent carbon dioxide atmosphere.

Analysis of immunofluorescence by fluorescence-activated cell sorting

The expression of CD44 on SW480, SW620 and SW707 was assessed by addition of 20 µl fluorescein isothiocyanate (FITC)-conjugated anti-CD44 monoclonal antibody (Mab) (Becton Dickinson, Mountain View, California, USA) to 100 µl cell suspension (1×10^6 cells/ml). FITC-conjugated isotype immunoglobulin G_1 mAb was used as a negative control. CD44 expression was analysed on a FACScan flow cytometer (Becton Dickinson) to detect the log of the mean channel fluorescence intensity with the acquisition of FL1. The minimum number of 10 000 events was collected and analysed on the software Lysis II (Becton Dickinson).

Assessment of tumour cell proliferation

SW480, SW620 and SW707 tumour cells were incubated in 96-well plates (Falcon, Lincoln Park, NJ, USA) ($0{\cdot}75 \times 10^4$ cells per well) and treated with either sodium hyaluronate 10–500 µg/ml (Pharmacia, Uppsala, Sweden) or carboxymethylcellulose 0·125–1·0 per cent for 24 h at 37°C in a humidified 5 per cent carbon dioxide atmosphere. Tumour cell proliferation was assessed using the commercially available 5-bromo-2-deoxyuridine labelling and detection kit III (Boehringer Mannheim Biochemica, Mannheim, Germany) on a Microtiter Plate Reader (Dynex Technologies, Chantilly, VA, USA) as per the manufacturer's instructions.

Assessment of tumour cell motility

Tumour cell motility was assessed using AC-48 well chemotaxis chambers (Neuro Probe, Gaithersburg, Maryland, USA). Briefly, 50 µl SW480, SW620 or SW707 tumour cell suspension was plated into the upper chamber (1×10^3 cells per well) and 25 µl phosphate-buffered saline, sodium hyaluronate or carboxymethylcellulose was added to the lower chamber. Tumour cells, which migrated through 8·0-µm polycarbonate membrane filters (Osmonics, Livermore, California, USA) and attached to 3·0-µm polycarbonate membrane filters, were stained with Rapi-Diff (DiacheM, Burscough, UK) and counted.

Measurement of peritoneal tumour cell growth *in vivo*

Adult male BD IX rats were randomized into two groups ($n = 11$ per group). Animals were anaesthetized with inhalation of halothane and a midline laparotomy was performed. In group 1 animals $0{\cdot}5 \times 10^6$ DHD/K12 tumour cells and 5 ml 0·4 per cent sodium hyaluronate in phosphate-buffered saline were transplanted into the peritoneal cavity, whereas rats in group 2 received $0{\cdot}5 \times 10^6$ DHD/K12 tumour cells and 5 ml phosphate-buffered saline into the peritoneal cavity. After 4 weeks all animals were killed. Intraperitoneal tumour nodules were visualized and counted directly with the aid of an operating microscope by two independent blinded observers.

Statistical analysis

All data are presented as mean(s.d.) and analysed by one-way analysis of variance. Differences were accepted as significant when $P < 0{\cdot}05$.

Results

CD44 receptor expression

SW480 tumour cells expressed high levels of CD44 receptors, whereas SW620 and SW707 had minimal expression of CD44 (*Fig. 1*).

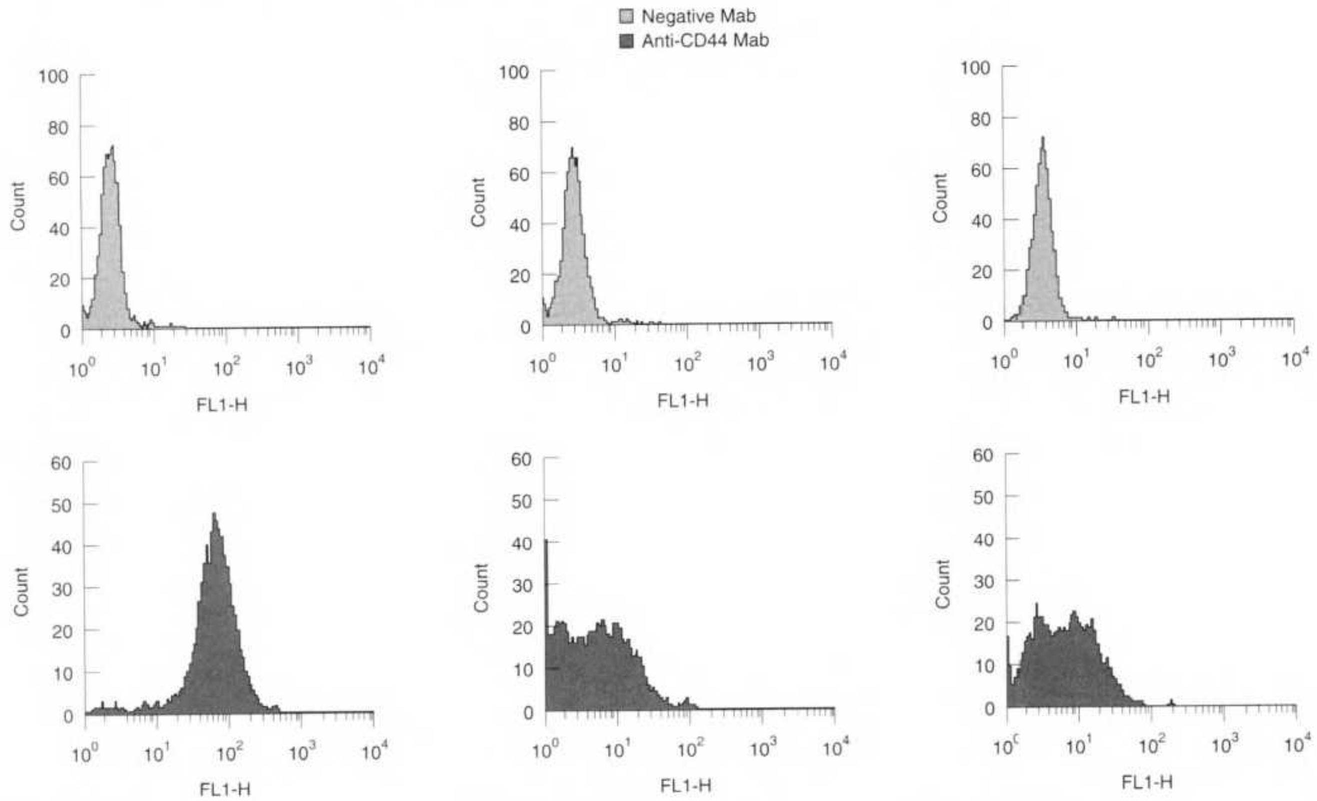
Effect of sodium hyaluronate on tumour cell proliferation and motility *in vitro*

Sodium hyaluronate significantly increased SW480 and SW707 tumour cell proliferation when the dosage exceeded 100 µg/ml ($P < 0{\cdot}05$ *versus* medium) (*Fig. 2*). SW620 tumour cells showed a significant increase in cell proliferation with a dose of sodium hyaluronate at 500 µg/ml ($P < 0{\cdot}05$ *versus* medium) (*Fig. 2*).

Treatment of SW480 tumour cells with sodium hyaluronate at 50 µg/ml resulted in a significant increase in tumour cell motility ($P < 0{\cdot}05$ *versus* medium) and there was a significant increase in SW480 tumour cell motility when the concentration of sodium hyaluronate reached 100 µg/ml ($P < 0{\cdot}01$ *versus* medium) (*Fig. 3*). Sodium hyaluronate also induced small but significant increases in tumour cell motility in SW620 and SW707 cells when the dose exceeded 100 and 500 µg/ml respectively ($P < 0{\cdot}05$ *versus* medium) (*Fig. 3*). Carboxymethylcellulose in doses ranging from 0·125 to 1·0 per cent had no significant effect on tumour cell proliferation and motility in SW480, SW620 and SW707 tumour cells.

In vivo experiments

The mean(s.d.) total tumour nodule count in the sodium hyaluronate group was significantly higher than that in the phosphate-buffered saline group (937(156) *versus* 438(109);



a SW480 **b** SW620 **c** SW707

Fig. 1 CD44 expression on SW480, SW620 and SW707. The cells were stained with fluorescein isothiocyanate (FITC)-conjugated negative monoclonal antibody (Mab) or FITC-conjugated anti-CD44 Mab and analysed by flow cytometry. FL1-H, fluorescence channel 1 height

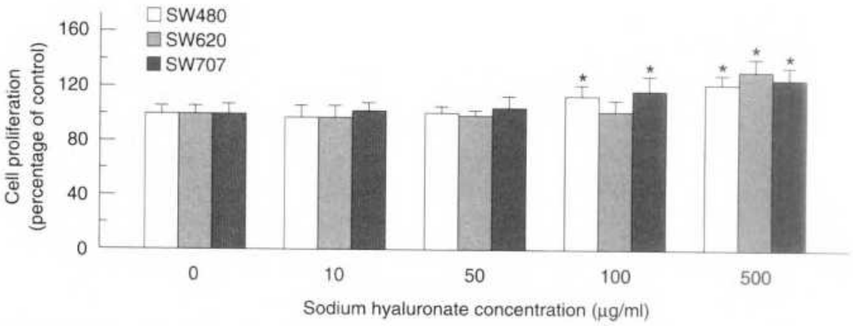


Fig. 2 Effect of sodium hyaluronate on tumour cell proliferation. SW480, SW620 and SW707 human colorectal cells were treated with different doses of sodium hyaluronate and cell proliferation was assessed as described in Materials and methods. Values are mean(s.d.) and representative of six separate experiments. $^*P < 0{\cdot}05$ *versus* treatment with phosphate-buffered saline (analysis of variance)

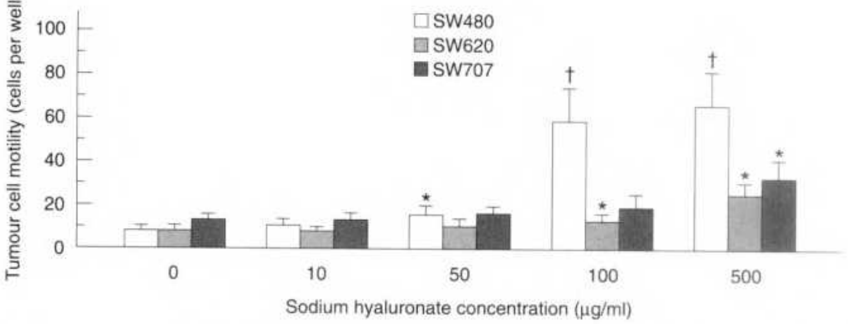


Fig. 3 Effect of sodium hyaluronate on tumour cell motility. SW480, SW620 and SW707 human colorectal cells were treated with different doses of sodium hyaluronate, and cell motility was assessed as described in Materials and methods. Values are mean(s.d.) and representative of three separate experiments. $^*P < 0{\cdot}05$, $^\dagger P < 0{\cdot}01$ *versus* treatment with phosphate-buffered saline (analysis of variance)

$P = 0{\cdot}016$). With regard to the different anatomical locations within the peritoneal cavity, there were higher tumour counts in the mesentery (146(32) *versus* 50(12); $P = 0{\cdot}012$) and omentum (160(31) *versus* 53(15); $P = 0{\cdot}069$) in rats exposed to sodium hyaluronate compared with those receiving phosphate-buffered saline.

Discussion

The development of tumour metastases is a multistep process. It involves a series of host–tumour cell interactions. General principles include angiogenesis followed by proliferation and detachment of the tumour cell. The tumour cell, with the aid of cell adhesion molecules, invades through the extracellular matrix with eventual entry into a blood vessel or lymphatic channel. It must then survive in the circulation and arrest in a capillary bed of an organ by adhesion to the endothelium. Invasion then occurs again and colonization of the secondary site occurs with proliferation[10].

Hyaluronic acid has an affinity for water and in its hydrated form is highly viscoelastic. These properties are thought to help provide a stable internal matrix in which cellular activities such as proliferation, migration and infiltration are promoted.

Sodium hyaluronate produced an increase in motility in colorectal tumour cells in a dose-dependent fashion and this appeared to be mediated by the CD44 receptor. This conclusion was based on the finding that SW480 cells, having expressed the highest level of baseline CD44, appeared to have a significant increase in motility at much lower doses of sodium hyaluronate. Moreover, with increasing doses of sodium hyaluronate, the motility of SW480 cells was markedly increased. Kubens and Zanker[11] showed that there is a higher level of CD44 receptors on SW480 than on SW620 cells. They also described the effect of sodium hyaluronate on the migration of these two cell lines. CD44 cannot be construed as the sole reason why SW480 tumour cells exhibited this increase in motility when exposed to hyaluronic acid. There are many other factors and receptors involved in cell motility. For example, increased metastatic potential was seen in certain colorectal tumour cells that were able to express the receptor for hyaluronic acid-mediated motility (RHAMM)[12]. There may also be other cell receptors expressed on these three human colorectal tumour cell lines that were not studied in this experiment.

On the other hand, whilst colorectal tumour cell proliferation was increased in a dose-dependent fashion by sodium hyaluronate, it did not appear to be dependent on CD44 receptor expression. It was surmised from this that CD44 does not appear to play a critical role in proliferation. Sodium hyaluronate may increase cell proliferation in a number of ways. It may provide a stable matrix in which colorectal tumour cells proliferate. Colorectal tumour cells may produce hyaluronidase, which breaks down hyaluronic acid, and the resultant oligosaccharides provide a stimulus for angiogenesis that is essential for tumour metastases[13]. Ropponen *et al.*[9] have also described increased potential for invasive growth and metastases when colorectal tumour cells synthesize their own hyaluronic acid. Hayen *et al.*[14] have shown that hyaluronan stimulates glioblastoma cell migration by modulating fibrin polymerization. The present experimental results also reconfirm that carboxymethylcellulose is an inert substance that does not appear to play any part in the metastatic process.

The *in vivo* experiments were performed mainly to support the *in vitro* results. Proliferation and motility are crucial components of the metastatic process and it was thought that, because tumour cell proliferation and motility were increased by sodium hyaluronate, hyaluronic acid would correspondingly increase metastatic growth. The *in vivo* results showed an overall significant increase in tumour load in the sodium hyaluronate group, confirming that sodium hyaluronate did increase the metastatic potential of colorectal tumour cells. A trend was observed in which there was a consistently higher tumour nodule count in the different components of the peritoneal compartment in the sodium hyaluronate group, with significance in the tumour nodule count in the greater omentum and mesentery.

It is concluded, therefore, that, although sodium hyaluronate acting as a barrier agent is efficient in preventing adhesions following surgery for benign disease, its use in patients undergoing operation for abdominal malignancy, in particular colorectal cancer, deserves further consideration and careful investigation.

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Original article

A normal appendix found during diagnostic laparoscopy should not be removed

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Background: Diagnostic laparoscopy has been introduced as a new diagnostic tool for suspected appendicitis. While the normal appendix used to be removed routinely, laparoscopy allows us to leave a normal looking appendix in place. This latter strategy is, however, not generally accepted. The long-term results of not removing a normal looking appendix were evaluated.

Methods: This was a prospective evaluation of 109 diagnostic laparoscopies for suspected appendicitis in which a normal looking appendix was left in place. After a median follow-up of 4·4 years a telephone questionnaire was performed.

Results: There were no false-negative laparoscopies. In 65 patients (60 per cent) another diagnosis was obtained (group 1). In 44 patients (40 per cent) no diagnosis was obtained (group 2). After a median interval of 8 months, 15 patients presented to the emergency department for symptoms possibly involving the appendix, during the median follow-up of 4·4 years. This resulted in readmission of nine patients, of whom eight were reoperated. In only one patient (1 per cent) was a histologically proven appendicitis found and the appendix removed. Some 105 patients were eligible for follow-up. Of the 100 patients interviewed (95 per cent), nine patients (9 per cent) (six in group 1 and three in group 2) still had recurrent pain in the right lower abdominal quadrant. There were no differences between patients with or without another diagnosis obtained during preceding laparoscopy.

Conclusion: It is safe to leave a normal looking appendix in place when a diagnostic laparoscopy for suspected appendicitis is performed, even if another diagnosis cannot be found at laparoscopy.

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Introduction

Clinical criteria used in the diagnosis of appendicitis lead to 15–30 per cent of normal appendices being removed at open operation[1–3]. New diagnostic tools such as ultrasonography and computed tomography have been introduced and achieve sensitivity rates of 75–89 per cent and specificity rates of 86–100 per cent for the diagnosis[4–7]. In the 'laparoscopic era', diagnostic laparoscopy has also been used because it is usually a simple procedure with a high specificity and has the possibility of confirming other diagnoses[8–11].

In the 'open era', the normal looking appendix found during exploration was removed routinely because the presence of the typical scar in the right lower abdominal quadrant might cause confusion about a future diagnosis. Since the introduction of diagnostic laparoscopy it has been suggested that an apparently normal appendix should be left in place, even if another diagnosis is not found[12,13]. Others report that a normal looking appendix found during laparoscopy can be removed safely because this does not increase postoperative morbidity or hospital stay[14]. Furthermore, the presence of an 'endoappendicitis', which might not be recognized during laparoscopy and could equally be missed at open operation, may lead to recurrent abdominal complaints and subsequent appendicectomy. This should justify the removal of an appendix that appears normal[15,16]. On the other hand, besides a prolonged operation time and costs, an increased number of complications after removal of a normal appendix has been reported[17–20]. Rarely, a normal appendix might also be useful in future reconstructive urological operations[21]. Considering the above aspects, the decision was made not to remove a normal looking appendix found during laparoscopy for suspected appendicitis. The present study was initiated to evaluate the long-term results of this strategy.

Patients and methods

Initial assessment

Consecutive patients referred to the hospital by general practitioners for suspected appendicitis were evaluated prospectively in the period 1994–1997.

Operative strategy

Diagnostic laparoscopy was performed in a selected group of patients, when there was doubt of the clinical diagnosis of appendicitis. Before laparoscopy metronidazole 500 mg was given intravenously. The appendix was removed only if appendicitis was confirmed during laparoscopy or when the appendix could not be interpreted clearly. A normal looking appendix was left in place even when no other explanation for the presenting abdominal symptoms could be found.

Follow-up

Patients were evaluated in the outpatient clinic 6 weeks after operation. Readmission, reoperation or new referrals for similar symptoms were recorded. Histological examination was performed in all patients in whom the appendix was removed.

In November 1999 a telephone questionnaire was performed. Patients were asked about remaining symptoms possibly involving the appendix, such as recurrent pain in the right lower abdominal quadrant, acute appendicitis and treatment by the general practitioner or other specialists.

Statistical analysis was performed with the SPSS computer program (SPSS, Chicago, Illinois, USA).

Results

Treatment and negative appendicectomy rates

There were 1050 patients (531 women (51 per cent), 389 men (37 per cent) and 130 children (12 per cent)) evaluated prospectively for suspected appendicitis. Of these, 202 patients (19 per cent) were eventually discharged without further treatment and 471 patients (45 per cent) underwent appendicectomy by muscle splitting incision, resulting in a negative appendicectomy rate of 13 per cent (61 of 471).

Some 377 patients (252 women (67 per cent), 83 men (22 per cent) and 42 children aged less than 11 years (11 per cent)) underwent diagnostic laparoscopy because there was doubt about the diagnosis of appendicitis. From this group 268 patients (71 per cent) eventually underwent appendicectomy, 94 patients laparoscopically and 174 patients by a muscle splitting incision. The negative appendicectomy rate in this group was 15 per cent (41 of 268). There was one complication following laparoscopy; superficial bleeding in an umbilical trocar opening was treated with a local suture.

Patients with a normal appendix found at laparoscopy

In 109 patients (29 per cent), a normal looking appendix was found during laparoscopy and left in place. Another diagnosis was obtained in 65 patients (60 per cent) (group 1), mostly gynaecological (48 patients; 74 per cent) (*Table 1*). When needed, appropriate treatment was initiated. In five patients laparoscopic adhesiolysis was performed and three patients with diverticulitis were treated conservatively. Gynaecologists treated nine patients with adnexitis with antibiotics, and three patients with ectopic pregnancy and four patients with adnexal torsion underwent ovariectomy. The remaining 15 patients received no further treatment. All patients recovered well.

In 44 patients (40 per cent) (group 2) no other diagnosis was obtained. These patients made an uneventful recovery without further therapy.

The median hospital stay was 2 days, 3 days for patients in group 1 and 2 days for those in group 2 ($P = 0{\cdot}2$; χ^2 test). No patient developed signs of (perforated) appendicitis in the postoperative period.

Follow-up

During follow-up 15 patients (14 per cent), eight in group 1 and seven in group 2, presented to the emergency

Table 1 Other diagnoses obtained by laparoscopy (group 1)

	No. of patients
Ovulation bleeding	13
Salpingitis	9
Ovarian cyst	9
Torsion of adnex	4
Mesenteric lymphadenitis	6
Retrograde menstruation	5
Endometriosis	5
Adhesions	5
Ectopic pregnancy	3
Diverticulitis	3
Other	3
Total	65

Forty-eight patients with gynaecological diagnosis department because of recurrent abdominal pain after a median time of 8 months. Nine of these, six patients in group 1 and three in group 2, were readmitted. One patient was discharged as the symptoms disappeared without further treatment.

In group 1, two patients were operated for suspected appendicitis; in one patient laparoscopy was performed in which a normal appendix was again left in place and one patient underwent appendicectomy. Two patients underwent an elective appendicectomy for chronic pain in the right lower quadrant. All appendices removed were normal and symptoms disappeared in all patients. One patient with signs of peritonitis underwent a laparotomy in which a perforated sigmoid was found and treated.

In group 2, one patient underwent an elective appendicectomy for chronic pain in the right lower abdominal quadrant in which a normal appendix was removed. Symptoms remained unchanged in this patient. Two patients underwent appendicectomy for suspected appendicitis; in one patient the appendix was normal and in one patient histologically proven appendicitis was found and the appendix was removed laparoscopically. There were no differences in recurrence of symptoms or treatment between groups 1 and 2.

After a median follow-up of 4·4 years a telephone questionnaire was performed. Three patients lived abroad and one patient had died from an unrelated disease so 105 patients were eligible for follow-up. One hundred patients (95 per cent) could be contacted, 59 patients in group 1 and 41 patients in group 2. Nine patients (9 per cent) still had episodes of recurrent pain in the right lower abdominal quadrant, six in group 1 and three in group 2. In group 1, two patients visited the general practitioner because of the pain, one patient an internist, one patient went to an emergency department elsewhere and two patients did not see another doctor. In none was another diagnosis obtained. The three patients in group 2 visited an internist, who diagnosed irritable bowel syndrome in two patients.

Discussion

The present study showed that it is safe to leave a normal looking appendix in place when diagnostic laparoscopy is performed for suspected appendicitis, even if no other pathology is found. No patient developed signs of peritonitis in the early postoperative period after laparoscopy, indicating that there were no false-negative laparoscopies. The symptoms mimicking appendicitis in patients in whom no pathology was found during laparoscopy disappeared without further treatment, so it is assumed that these symptoms were mainly due to a self-limiting disease, such as gastroenteritis. If an entity such as endoappendicitis exists it was of no clinical importance in this study population. The endoappendicitis was either cured by a single dose of metronidazole 500 mg given at laparoscopy or did not need further therapy.

During long-term follow-up an inflamed appendix was removed in only one patient (1 per cent). The chance of developing appendicitis was no higher than that in a normal population so there is no rationale for a 'prophylactic appendicectomy'.

Symptoms disappeared in three of the five patients whose histologically normal appendix was removed during the second admission, but in one patient the symptoms remained unchanged and in one patient the symptoms disappeared after the appendix was again left in place during diagnostic laparoscopy. Therefore it remains doubtful whether the disappearance of symptoms in the three patients was in any way related to the appendicectomy.

Ninety-one (91 per cent) of the interviewed patients were symptom free after a median follow-up of 4·4 years. It remains uncertain whether the pain in the right lower quadrant in the remaining nine patients was caused by the appendix. Their symptoms might also have been caused by other diseases such as irritable bowel syndrome.

There were no differences in recurrent symptoms or eventual treatment between patients in whom another diagnosis was made during laparoscopy and patients in whom no pathology was found during laparoscopy. It does not therefore seem justified to remove a normal looking appendix only when no other pathology is found during laparoscopy, as suggested previously[22].

From the above results, the most valid reason for removing a normal looking appendix would be to avoid future diagnostic confusion in case of acute abdominal pain. This does not weigh favourably against the disadvantages of appendicectomy, such as adhesion formation leading to possible future small intestinal obstruction and extra costs of (laparoscopic) equipment and histopathological examination.

From the present study it can be concluded that diagnostic laparoscopy for suspected appendicitis is a safe procedure that is helpful in obtaining other diagnoses. It also appears to be justifiable and possibly even preferable to leave the appendix in place as recurrent appendicitis and the need for surgery during follow-up is uncommon.

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Acute-phase protein response, survival and tumour recurrence in patients with colorectal cancer

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Introduction: An acute-phase protein response (APPR) has been associated with reduced crude survival rates and increased recurrence following apparently curative resection in patients with colorectal cancer. This study investigated the prognostic significance of a preoperative and postoperative APPR in relation to disease-specific mortality rate.

Methods: Some 202 patients with colorectal cancer were followed for at least 5 years. C-reactive protein concentration, measured before and at 3 months after operation, was used as an index of the APPR. Univariate and multivariate analyses were performed on a number of potential prognostic factors.

Results: Thirty-six per cent of patients had an APPR and this was associated with a higher rate of local tumour invasion, fewer curative resections and a higher carcinoembryonic antigen (CEA) concentration. There was no difference in Dukes' stage between patients with or without an APPR. The most important prognostic factor related to both disease-specific and crude survival was Duke's stage ($P < 0.0001$). Subgroup analysis demonstrated that APPR had prognostic significance only in patients with advanced disease ($P = 0.013$). An APPR was present in a minority of patients (11 per cent) after operation and was not associated with increased likelihood of tumour recurrence.

Conclusion: The APPR is increased in more than a third of patients presenting with colorectal cancer and is associated with more frequent local tumour invasion, fewer curative resections and a higher CEA level. An APPR at 3 months after operation does not have the prognostic significance reported by earlier studies.

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Introduction

The acute-phase proteins are a family of proteins synthesized by the liver whose concentrations change in response to infection, injury and neoplasia[1]. In response to injury the concentrations of some acute-phase proteins, such as C-reactive protein (CRP), serum amyloid A, fibrinogen and α-1 antichymotrypsin, increase. In contrast, levels of others, such as albumin, prealbumin and transferrin, fall. These changes are believed to be mediated by proinflammatory cytokines, notably the hepatocyte-stimulating cytokine interleukin (IL) 6[2]. In infection or acute injury acute-phase proteins function as opsonins, antiproteases and chelating agents to facilitate the acute inflammatory response and limit tissue injury. However, if inappropriately prolonged or severe, the acute-phase protein response (APPR) may be detrimental in that reprioritization of nitrogen metabolism away from peripheral tissues and towards the liver may lead to accelerated wasting and shortened survival.

In both lung and pancreatic cancer the presence of an increased APPR is associated with reduced duration of survival irrespective of stage of disease[3,4]. A similar reduction in survival time associated with an APPR has been observed in patients with advanced renal cancer[5] and lymphoma[6]. Studies in patients with colorectal cancer have reported an association between preoperative CRP concentration and the presence of metastatic disease or local peritoneal involvement[7]. In the same study it was demonstrated that patients with a raised serum CRP concentration had a poorer prognosis than those whose CRP level was not increased. Another smaller study has reported that detection of an APPR following curative resection of colorectal cancer indicates patients who subsequently develop tumour recurrence[8]. Heys *et al.*[9] have shown that a low preoperative plasma albumin concentration (which can reflect an ongoing APPR) is also an indicator of poor prognosis in patients with colorectal cancer.

Clearly it is not possible to be certain about the mechanism linking an acute-phase response with shortened survival in any one group of patients. On the one hand, shortened survival might be related to accelerated cachexia (see above). On the other, it may relate to the phenotype of the tumour and hence its growth characteristics or metastatic potential. Indeed it may involve a combination of factors. Independent of such arguments, none of the above studies that have investigated the APPR in cancer has looked at disease-specific deaths. Rather deaths from all causes have been included. Since the hypothesis is that the APPR may be a manifestation of an adverse host–tumour interaction (by whatever mechanism) and may therefore be associated with a reduced survival time, it would seem logical to study disease-specific death.

The purpose of the present study was to investigate the prognostic significance of both the preoperative and postoperative APPR in a substantial cohort of patients with colorectal cancer in relation to disease-specific mortality rate.

Patients and methods

The study group comprised a cohort of 202 patients with colorectal cancer who underwent tumour resection in South East Scotland between November 1991 and December 1992. Data were collected prospectively, by a dedicated research nurse, as part of the Lothian Large Bowel Cancer Study and permission to access data was obtained from the steering committee of this study. The study was approved by the Lothian Regional Ethics Committee. The mode of presentation, tumour stage and site were recorded in all cases. Preoperative serum measurements of albumin, CRP and carcinoembryonic antigen (CEA) were recorded in all cases. In addition 3-month postoperative CRP and CEA measurements were available in 88 and 113 patients respectively. All patients have been followed up until death or for a minimum of 5 years. Cause of death was ascertained on the basis of all information recorded on death certificates.

Sample sizes

Sample sizes were calculated based on previous studies of the APPR in colorectal cancer. The study was designed to be able to demonstrate a 20 per cent difference in survival at 2 years dependent on preoperative CRP concentration in patients undergoing curative resection of a colorectal cancer with 95 per cent confidence intervals and 90 per cent power, assuming that the smaller probability of survival would be 50 per cent. Similarly, with respect to the previously reported association between postoperative CRP concentration and tumour recurrence, the study could detect a 50 per cent difference in tumour recurrence within 5 years with 95 per cent confidence intervals and 90 per cent power assuming that the smaller probability was 10 per cent.

Statistical analysis

Patients were divided into those with a CRP level of 10 mg/l or above or a level below 10 mg/l based on previous studies[4,7]. In addition to the above conventional definition of the APPR used to designate those with or without an APPR based on CRP concentration, a cut-off level of CRP of 5 mg/l was used to enable comparison with the study on tumour recurrence reported by McMillan *et al.*[8]. Comparisons between groups of patients were performed using either Mann–Whitney *U* test or contingency table analysis (χ^2 test) as appropriate. Survival curves were plotted using the Kaplan–Meier technique and statistical differences were analysed using the log rank test. A multivariate analysis was performed using Cox's proportional hazards model. Concentrations of CRP and CEA were not normally distributed and were therefore analysed as categorical variables using log minus log plots to confirm that this was a valid approach and that the constancy of the hazard was maintained. Other covariates included in the analysis were age, sex, albumin concentration and Dukes' stage. Survival was calculated from the time of resection of colorectal cancer and deaths not due to colorectal cancer were censored.

Results

Patient characteristics

Patient characteristics are given in *Table 1*. There were 96 men and 106 women of median age 72 (range 33–94) years. Thirty-two per cent of patients presented as an emergency, most frequently because of colonic obstruction. The resection was considered curative by the operating surgeon in 125 patients (62 per cent). Nineteen patients (9 per cent) had local fixity of the tumour, 32 patients (16 per cent) had liver metastases, and 27 (13 per cent) had confirmed and five had suspected distant metastases at other sites. The median (range) albumin concentration for patients by tumour stage was Dukes' A 39 (32–47) g/l, Dukes' B 35 (16–44) g/l, Dukes' C 36 (19–45) g/l and Dukes' 'D' 35 (19–45) g/l.

Table 1 Characteristics of patients with colorectal cancer ($n = 202$)

Age (years)*	72 (33–94)
Sex ratio (M : F)	96 : 106
Dukes' stage	
A	17 (8)
B	99 (49)
C	54 (27)
'D'	32 (16)
Site	
Caecum/ascending	67 (33)
Transverse colon	5 (2)
Descending colon	18 (9)
Sigmoid colon	26 (13)
Rectum	86 (43)
Presentation	
Elective	137 (68)
Emergency	65 (32)
Fixed tumour	19 (9)
Liver metastases	32 (16)
Lymph nodes	39 (19)
Peritoneal metastases	6 (3)
Local invasion	34 (17)
Curative resection	125 (62)

*Values are median (range); otherwise values in parentheses are percentages

Preoperative C-reactive protein and clinical characteristics

There were 130 patients with a preoperative CRP concentration below 10 mg/l and 72 in whom the CRP was 10 mg/l or more (*Table 2*). No differences were evident between patients with a normal or raised CRP level with respect to age, sex, Dukes' stage, proportion with liver metastases or site of tumour. More patients with a raised CRP concentration had local tumour invasion ($P < 0.005$) and fewer underwent curative resection of the colorectal tumour ($P < 0.005$). Patients with a raised CRP concentration had a significantly higher concentration of CEA ($P = 0.004$) and lower concentration of albumin ($P < 0.001$) than patients with a normal preoperative CRP concentration.

Causes of death

There were 11 postoperative deaths (5 per cent) and, of the surviving 191 patients, 100 subsequently died during follow-up. Of these, 23 were deaths not due to colorectal cancer and the most frequent cause of death was cardiovascular disease (ruptured aortic aneurysm, three; cerebrovascular accident, five; myocardial infarction or arrhythmia, four; heart failure, one; and mesenteric ischaemia, one). The remaining deaths not due to colorectal cancer were from trauma (two patients), acute pancreatitis (one), renal failure (one) or other malignancy (one each of bladder, prostate, gastric carcinoma, mesothelioma and myelodysplastic disease). Of these 23 deaths not due to colorectal cancer, colorectal cancer was reported as being present or as a contributing factor leading to death in six patients. All remaining deaths were attributed to colorectal cancer.

C-reactive protein and disease-specific survival time

Univariate analysis of all patients showed that Dukes' stage, low albumin concentration (less than 35 g/l), raised CEA level (above 60 units/l) and raised CRP concentration (10 mg/l or more) were associated with reduced disease-specific survival (*Table 3*). Taking other variables into consideration in a multivariate analysis, Dukes' stage ($P < 0.0001$) and high CEA level ($P = 0.0028$) remained significant covariates. Age and sex were non-significant in both univariate and multivariate analyses.

To investigate the potential effect of the APPR further, Dukes' stage was removed from the model and the analysis was performed in patients with Dukes' stage A or B tumours and C or 'D' tumours separately (*Table 4*). In patients with Dukes' A and B tumours, albumin concentration less than 35 g/l ($P = 0.024$) and CEA concentration above 60 units/l ($P = 0.0085$) were significantly associated with shorter disease-specific survival. In Dukes' C and 'D' tumours CEA and albumin became non-significant and the only covariate significantly associated with shorter disease-specific survival was preoperative CRP concentration ($P = 0.013$).

C-reactive protein and crude survival

Multivariate analysis based on crude survival demonstrated that when all patients were considered Dukes' stage remained the most significant factor associated with poor prognosis ($P < 0.0001$). An albumin concentration of below 35 g/l ($P = 0.025$) and CEA level above 60 units/l ($P = 0.048$) were also significantly associated with survival. Considering patients with Dukes' stage A and B tumours, the only factor associated with poor prognosis was albumin concentration below 35 g/l ($P = 0.028$). In patients with Dukes' C and 'D' tumours, albumin had no prognostic significance. However, a CRP concentration of 10 mg/l or more ($P = 0.038$) and CEA level above 60 units/l ($P = 0.031$) were associated with reduced crude survival.

Postoperative C-reactive protein and tumour recurrence

Measurement of CRP at 3 months after surgery was undertaken in 88 patients. Only four of these patients (5 per cent) had a CRP level of 10 mg/l or more and ten (11 per

Table 2 Characteristics of patients with serum C-reactive protein concentration above and below 10 mg/l

	CRP < 10 mg/l (n = 130)	CRP ≥ 10 mg/l (n = 72)	χ^2	P
Age (years)*	72 (36–94)	72 (33–92)	—	0·49‡
Sex ratio (M : F)	64 : 66	32 : 40	—	0·42
Site			1·78	0·76
Caecum/ascending	35	32		
Transverse	4	1		
Descending	11	7		
Sigmoid	16	10		
Rectum	57	29		
Liver metastases†	16 (12)	16 (22)	3·40	0·071
Local invasion†	10 (8)	24 (33)	21·84	< 0·005
Dukes' stage			4·40	0·28
A	13	4		
B	64	35		
C	37	17		
'D'	16	16		
Preoperative CEA (units/l)*	66 (< 30–9200)	163 (30–80 000)	—	0·004‡
Preoperative albumin (g/l)*	37 (18–47)	32 (16–46)	—	< 0·001‡
Curative resection	76	49	15·80	< 0·005

*Values are median (range); †values in parentheses are percentages; ‡Mann–Whitney U test. CRP, C-reactive protein; CEA, carcinoembryonic antigen

Table 3 Results of univariate and multivariate analyses of factors potentially associated with prognosis in 202 patients with colorectal cancer

	P	
	Univariate	Multivariate
Age	0·434	0·191
Sex	0·063	0·259
Albumin	0·0028	0·075
CEA	0·0001	0·0028
CRP	0·0048	0·142
Dukes' stage	<0·0001	<0·0001

Analysis was performed on the basis of disease-specific colorectal cancer deaths. CEA, carcinoembryonic antigen; CRP, C-reactive protein

Table 4 Results of univariate and multivariate analyses of factors potentially associated with prognosis in patients with Dukes' stage A or B and C or 'D' colorectal cancer

	P			
	Dukes' A and B (n = 116)		Dukes' C and 'D' (n = 86)	
	Univariate	Multivariate	Univariate	Multivariate
Age	0·138	0·158	0·834	0·762
Sex	0·708	0·785	0·283	0·455
Albumin	0·073	0·024	0·062	0·279
CEA	0·001	0·008	0·01	0·092
CRP	0·35	0·416	0·001	0·013

Analysis was performed on the basis of disease-specific colorectal cancer deaths. CEA, carcinoembryonic antigen; CRP, C-reactive protein

cent) had a CRP concentration of 5 mg/l or more. Tumour recurrence developed in four of ten patients with a CRP concentration of 5 mg/l or above and 28 of 78 patients with CRP level below 5 mg/l ($\chi^2 = 0{\cdot}067$, d.f. = 1, $P = 0{\cdot}84$).

Discussion

The interaction between tumour and host metabolism has the potential to influence certain aspects of a patient's clinical course. In pancreatic cancer approximately half of patients have an APPR at the time of diagnosis, characterized by high serum CRP concentration and hypoalbuminaemia[4]. This APPR is associated with poor prognosis and appears to be independent of other conventional prognostic factors, such as age, sex and stage of disease[4]. In some forms of the anorexia–cachexia syndrome, an ongoing APPR may be regarded as a marker of proinflammatory cytokine-driven metabolic changes leading to accelerated weight loss and early demise. Colorectal cancer is, however, less frequently associated with weight loss or obvious alterations in metabolism particularly at the time of presentation, although patients with advanced disease may become cachectic[10]. In the present study 36 per cent of patients had a CRP concentration of greater than 10 mg/l (similar to the 41 per cent reported by Nozoe and coworkers[7]). Moreover, following resection of the primary tumour, the proportion of patients with a CRP concentration of 10 mg/l or more was reduced to 5 per cent. This suggests that the presence of the primary tumour is associated either directly or indirectly with CRP production. The mechanism of this effect is unknown but acute-phase protein production is known to be mediated by proinflammatory cytokines such as IL-6[11], tumour necrosis factor, IL-1 and IL-8[12]. It is likely that either the primary tumour, or the inflammatory response associated with the tumour, is responsible for cytokine production.

In a previous study by Nozoe *et al.*[7], patients with a raised CRP concentration before resection of a colorectal primary tumour were found to have more advanced disease. In the present study a raised preoperative CRP concentration was associated with a higher frequency of local invasion, non-curative resection and a higher CEA concentration. This would seem to suggest that patients with a raised CRP level might indeed have disease at a more advanced anatomical stage. However, there was no difference in Dukes' stage or proportion of patients with liver metastases between patients with or without a raised serum level of CRP (*Table 2*). Taken together, these findings suggest that the APPR might reflect a more bulky primary tumour mass but not metastatic disease. Clearly a large tumour mass is more prone to undergoing central necrosis which in turn would elicit a local and systemic inflammatory response and hence an APPR. Further studies are required to resolve this issue.

Nozoe *et al.*[7] also reported a significant association between raised preoperative CRP concentration and survival; the 2-year survival rate for patients with a raised CRP level was 47 per cent compared with 90 per cent for those with no increase in CRP concentration. Performing similar univariate analysis on the present data 2-year survival estimates of 44 and 66 per cent were obtained for patients with a raised or normal preoperative CRP concentration respectively. In the present study, however, the discriminatory value of CRP in predicting prognosis was diminished to the point at which it no longer retained statistical significance when other factors, including Dukes' stage and CEA concentration, were considered. The statistical model demonstrated that the most important prognostic factor was tumour stage, and so the analyses were repeated to determine whether the acute-phase response influenced survival stage for stage. This analysis demonstrated that CRP was of prognostic importance only in patients with Dukes' C and 'D' tumours.

In the present study the reported association between the APPR following apparently curative tumour resection and subsequent tumour recurrence was evaluated further. Only 11 per cent of patients had a CRP level of 5 mg/l or more 3 months after tumour resection compared with the 42 per cent reported by McMillan *et al.*[8]. In the latter study 73 per cent of patients with a CRP concentration above 5 mg/l and 10 per cent of those with a lower concentration developed tumour recurrence compared with recurrence rates of 40 (four of ten patients) and 36 per cent (28 of 78) in the present study. In the pilot study reported by McMillan and coworkers the sample size was probably too small to achieve statistical power[8]. In the present, much larger study the similarity in recurrence rates between patients with and without a raised level of CRP suggests that any effect of CRP on tumour recurrence is likely to be small. An alternative explanation may be that the patients in the earlier study had larger tumours at the time of resection and may have been more predisposed to developing tumour recurrence.

The present study has demonstrated that a third of patients with colorectal cancer have a raised CRP concentration at the time of presentation. The presence of an increased APPR did not have the degree of prognostic significance reported in earlier studies in colorectal cancer. Furthermore, an APPR was also associated with more frequent local tumour invasion, fewer curative resections and a higher CEA concentration. Whether tumours associated with an APPR are characterized predominantly by such a pattern of behaviour or by the ability to elaborate hepatocyte-stimulating cytokines, or by the development of cachexia remains to be determined.

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Influence of aspirin on early allograft thrombosis and chronic allograft nephropathy following renal transplantation

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Background: Primary thrombosis and chronic allograft nephropathy are important causes of early and late graft loss, respectively, following renal transplantation. This study examined the potential for aspirin therapy to reduce these complications.

Methods: A consecutive series of 105 cadaveric renal transplants treated with aspirin 150 mg daily for the first 3 months after transplantation was compared with an untreated historical control group ($n = 121$). Protocol needle-core biopsies were performed on all transplants in both groups at 1 week and 12 months after transplantation. Needle-core allograft biopsies were performed at 3, 6 and 12 months after transplantation, and serum creatinine was measured at each outpatient attendance for the duration of follow-up.

Results: There was a significantly lower rate of primary allograft thrombosis in patients treated with aspirin (none of 105) compared with that in the control group (six (5 per cent) of 121; $P = 0{\cdot}03$). There were no differences in renal function or 2-year allograft survival between the two groups. Aspirin-treated patients had a lower incidence of chronic allograft nephropathy at 1 year than controls although this did not reach statistical significance (16 *versus* 26 per cent; $P = 0{\cdot}075$). There were no major bleeding complications in either group in association with peptic ulcer disease or following renal transplant biopsy.

Conclusion: Aspirin reduced the rate of early graft thrombosis of renal transplants in this series but did not improve renal function or graft survival. A trend towards a lower rate of chronic allograft nephropathy was noted with aspirin treatment. These findings require confirmation in a prospective randomized trial.

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Introduction

Primary renal allograft thrombosis and chronic allograft nephropathy (CAN) are important causes of early and late graft loss in renal transplantation respectively. Primary allograft thrombosis is uncommon, occurring in approximately 1–8 per cent[1–6] of renal transplants, but it accounts for a high proportion of early graft loss as the incidence of graft failure secondary to acute irreversible rejection has declined following the introduction of cyclosporin[7]. Conversely, long-term graft survival has remained largely unchanged and CAN, characterized by the development of allograft vasculopathy and progressive glomerular and interstitial fibrosis, accounts for over 40 per cent of graft failures after 1 year[8].

Aspirin (acetylsalicylic acid) is widely used in vascular medicine as an antithrombotic agent[9]. It acts by inhibiting the enzyme cyclo-oxygenase, reducing production of thromboxane A_2 by circulating platelets (a potent platelet aggregator and vasoconstrictor), and reducing the production of prostacyclin (a vasodilator) by vascular endothelium[10]. Low and moderate doses of aspirin (75–150 mg) are thought to affect circulating platelets to a greater degree than vascular endothelium, thereby reducing platelet aggregation and thrombosis[10]. In organ transplantation, aspirin reduces allograft thrombosis rates following liver transplantation in humans[11] as well as in renal allografts in animals[12]. In addition, inhibition of eicosanoid production has also been shown to attenuate the severity of renal ischaemia–reperfusion injury and the development of allograft nephropathy in animal models[13–15]. Aspirin therapy therefore has the potential reduce both early and late graft loss following human renal transplantation, although to date the effect of aspirin on renal allograft outcome has not been reported.

Aspirin was introduced as a routine part of the transplant protocol in Leicester in 1993. This report is a retrospective analysis of the influence of this change on the incidence of primary graft thrombosis and chronic rejection following renal transplantation.

Patients and methods

A consecutive series of 226 heart-beating donor cadaveric renal transplants performed during the period 1990–1995 was studied. During the 3-year interval 1993–1995, 105 transplants were performed and all patients received oral aspirin 150 mg daily, starting on the first day after transplantation and continuing for 3 months. Patients transplanted in the previous 3-year interval 1990–1992 (*n* = 121) did not receive aspirin and were analysed as a historical control group. All patients in the later series also received oral ranitidine 150 mg twice daily for the first 3 months after transplantation to prevent peptic ulcers, and subcutaneous heparin 5000 units before and 5000 units twice daily for 5 days following transplantation, as prophylaxis against thromboembolism.

Technical considerations and patient risk factors (*Table 1*)

The renal vein was anastomosed to the external iliac vein in all patients. The choice of arterial anastomosis depended on the presence or absence of a suitable donor aortic Carrel patch, but there were no statistical differences in arterial anastomotic technique between the aspirin and control groups. Recipient age was significantly higher in the aspirin compared with the control group but there were no statistically significant differences in the number of renal arteries, donor age, cold ischaemic time, anastomosis time and number of DR mismatches between the two groups. There was no difference in haematocrit between the two groups either before operation or at 6 months after transplantation (*Tables 1* and *2*).

Table 1 Technical considerations and risk factors in patients undergoing renal transplantation

	Aspirin (*n* = 105)	Control (*n* = 121)	*P*
Arterial anastomosis			0·33*
External iliac artery	89	108	
Internal iliac artery	16	13	
Renal arteries			0·83*
Single	12	12	
Multiple	93	109	
Recipient age (years)	43(11)	40(10)	0·03†
Donor age (years)	37(10)	35(12)	0·18†
Cold ischaemic time (h)	20(8)	21(7)	0·32†
Anastomosis time (min)	31(8)	32(6)	0·29†
HLA-DR mismatch	0·9(0·7)	1·0(0·6)	0·25†
Haematocrit (%)	30(5)	31(6)	0·08†

Values are mean(s.d.). HLA, human leucocyte antigen. *Fisher's exact test; †Student's *t* test

Immunosuppression protocol

Patients in both the aspirin and control groups were immunosuppressed with cyclosporin (Sandimmune®; Sandoz Pharmaceutical, Basel, Switzerland) as either dual therapy with prednisolone or triple therapy with prednisolone and azathioprine. Cyclosporin was given at an initial dose of 15–17 mg per kg per day (in divided doses twice daily) and this was reduced weekly by 2 mg per kg per day until a baseline dosage of 5 mg per kg per day was reached. Trough whole blood levels of cyclosporin were measured by high-performance liquid chromatography before 1994 and monoclonal antibody (TDx®; Abbott Laboratories, Wiesbaden, Germany) thereafter, and doses adjusted to achieve levels of 200–300 ng/ml in the first 3 months after transplantation and 100–200 ng/ml thereafter.

All patients received oral prednisolone 20 mg/day for 3 months and this dose was then tapered in a linear fashion down to a maintenance dose of 10 mg on alternate days by 6 months after transplantation. Azathioprine was administered as a single daily dose of 1–2 mg/kg, adjusted according to the peripheral white cell count.

Table 2 Early graft function, rejection rates, renal function and cyclosporin levels

	Aspirin (*n* = 105)	Control (*n* = 121)	*P*
Delayed graft function*‡	35 (33)	36 (30)	0·57¶
Acute rejection*§	32 (30)	46 (38)	0·26¶
GFR at 1 year (ml/min)†	47(4)	43(3)	0·42**
Serum creatinine at 2 years (µmol/l)†	172(8)	183(10)	0·40**
Cyclosporin level (ng/ml)†			
1 year	182(105)	277(139)	< 0·001**
2 years	141(49)	257(166)	< 0·001**
Haematocrit at 6 months (%)†	37(6)	39(6)	0·18**

*Values in parentheses are percentages; †values are mean(s.d.); ‡Need for dialysis in the first week after transplantation; §All confirmed by needle-core biopsy; ¶Fisher's exact test; **Mann–Whitney *U* test. GFR, glomerular filtration rate

Monitoring of outcome

Needle-core allograft biopsies were performed at 3, 6 and 12 months after transplantation. The 12-month biopsy was used to define the presence of CAN. Grafts with delayed function were biopsied weekly until graft function occurred. All episodes of acute allograft dysfunction (creatinine rise of more than 30 µmol/l or over 10 per cent) were also investigated by needle-core biopsy. Renal function was monitored by serum creatinine estimations at all routine clinic visits and isotope glomerular filtration rate (GFR) measurements (^{51}Cr-radiolabelled ethylenediamine tetra-acetic acid) 3, 6 and 12 months after transplantation.

Data collection and statistical analysis

Data were collected prospectively in a computerized database and are presented as mean(s.d.). Differences between groups were evaluated by Student's *t* test for continuous variables and Fisher's exact test for categorical variables. All tests were two-tailed and statistical significance was taken as $P<0.05$. Graft survival curves were constructed using the Kaplan–Meier method, defining death with a functioning transplant as graft failure; differences were compared using the log rank test. Delayed graft function was defined as the need for dialysis in the first week after transplantation, with the exception of a single dialysis on the first postoperative day when indicated specifically for hyperkalaemia. Rejection episodes were diagnosed using clinical and biochemical parameters but confirmed in all patients by needle-core biopsy. CAN was defined as progressive renal dysfunction associated with proteinuria and biopsy evidence of allograft vasculopathy, and glomerular and interstitial fibrosis.

Results

Allograft thrombosis rates

There were six graft thromboses (five venous and one arterial) in the control group (5 per cent), and all six kidneys were lost as a result. There was no thrombosis in the aspirin-treated group ($P=0.03$, Fisher's exact test).

Early graft function, rejection rates and renal function

A lower rate of acute rejection was recorded in the aspirin group, but this did not reach statistical significance ($P=0.26$) (*Table 2*). In addition, there was no significant difference in the incidence of delayed graft function, GFR at 1 year or serum creatinine level at 2 years after transplantation between the two groups. Cyclosporin levels were significantly lower in the aspirin-treated group at 1 and 2 years.

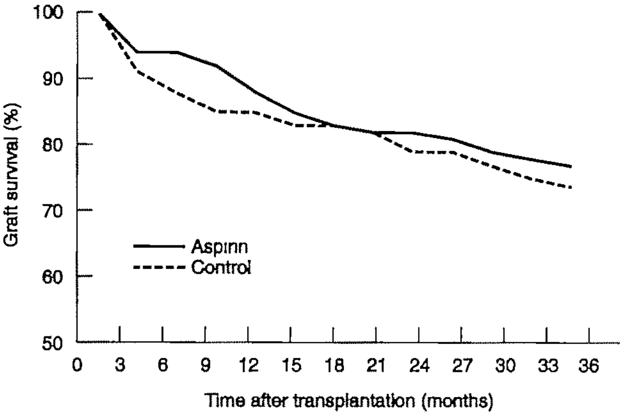

Fig. 1 Renal allograft survival in aspirin and control groups

Graft survival and chronic allograft nephropathy

There was no difference in graft survival between the aspirin and control groups at 2 years (*Fig. 1*). The rate of biopsy-proven CAN was lower in the aspirin-treated group (16 per cent) than in controls (26 per cent), but this finding was of marginal statistical significance only ($P=0.075$).

Aspirin-related complications

Aspirin was well tolerated by the majority of patients. The drug was stopped earlier than planned in two patients with peptic ulcer disease. The incidence of macroscopic haematuria following percutaneous needle-core renal transplant biopsy was 9 per cent in the aspirin-treated group and 7 per cent in the control group (*P* not significant, Fisher's exact test).

Discussion

These data demonstrate that aspirin prophylaxis significantly reduced the incidence of renal allograft thrombosis in comparison to the period before the introduction of aspirin. This study also raises the possibility that aspirin treatment in the first 3 months after transplantation may reduce the development of CAN.

The analysis was limited in that it involved relatively small numbers and the use of a historical control group. As this was a single-centre study, variations in surgical technique associated with multicentre studies were eliminated. One possible source of bias was that an increased awareness of the risks of renal vein thrombosis which coincided with the introduction of aspirin may have led to a modification of surgical technique after 1993. In addition, the lower cyclosporin levels in the aspirin-treated group may have influenced the long-term results, reflecting more rapid dose reductions with increasing experience. This was not, however, manifest by significantly improved serum creatinine level or GFR in the aspirin-treated group and, given the variable bioavailability of Sandimmune cyclosporin with broad confidence intervals in all groups studied, its effect on outcome are difficult to measure. In the absence of prospective randomized controlled trials, retrospective data provide the only information available to guide practice. This is the best evidence to date that aspirin may improve early and late graft outcome.

As reported in this study, primary renal allograft thrombosis is most commonly due to thrombosis of the renal vein rather than the transplant artery[3–6]. Most thromboses occur within 2 weeks of transplantation and the commonest outcome is loss of the graft[1–4]. The importance of renal vein thrombosis is underscored by the fact that it is frequently associated with allograft rupture, a potentially life-threatening emergency[7]. In the present study, before the introduction of aspirin, allograft thrombosis rates were comparable to those reported by others[1–4] and led to graft loss in each case. The abolition of allograft thrombosis with the introduction of aspirin at a dose of 150 mg once daily, without any increase in adverse side-effects, may therefore represent an important step in reducing early graft loss. Whether lower cyclosporin levels in the aspirin-treated group influenced the rate of allograft thrombosis is unclear. There is evidence to suggest that cyclosporin may affect haemostasis. Cyclosporin increases platelet activation, endothelial cell toxicity and thromboxane A_2 production *in vivo* and *in vitro*, as well as decreasing prostacyclin levels in transplant patients[16–19]. Clinical studies comparing the incidence of allograft thrombosis in patients on cyclosporin and FK506 or azathioprine-based regimens have produced conflicting results. An association between allograft thrombosis and cyclosporin-based therapy is unproven[3–6,20,21]. Intermittently high levels of cyclosporin in combination with other risk factors for allograft thrombosis, such as hypovolaemia or acute rejection[1–3], may potentially combine to produce allograft thrombosis in the immediate postoperative period. It is, however, impossible to detect such circumstances from retrospective data. The current study suggests that aspirin at least partly reverses renal allograft thrombogenicity, regardless of its aetiology. This may be mediated by its anti-inflammatory and antithrombotic effects on renal eicosanoid production[22–24], although reduced cyclosporin nephrotoxicity via a reduction in renal thromboxane synthesis may be significant[24,25].

CAN, the leading cause of late renal transplant loss[8], occurs in response to a combination of immunological and non-immunological factors (including ischaemia–reperfusion injury and choice of immunosuppression). The cellular and molecular mechanisms responsible for CAN are thought to be initiated at an early stage after transplantation[8] and changes in intrarenal gene expression associated with subsequent graft fibrosis are evident as early as 6 months after transplantation[26]. The data presented here suggest that a moderate dose of aspirin, administered for the first 3 months after transplantation, may attenuate these changes. The long-term significance of the reduced incidence of CAN at 1 year, along with lower cyclosporin levels and the absence of any difference in renal function or graft survival at 2 years, requires further follow-up. Higher cyclosporin levels, in particular in the non-aspirin-treated group, are thought to be a risk factor for CAN[8].

There is abundant clinical and experimental evidence to suggest that eicosanoids are important in the development of CAN, although their precise role in its pathogenesis is unclear. The administration of cyclo-oxygenase inhibitors, such as indomethacin[27], thromboxane inhibitors[28] and antiplatelet agents[29], has been shown to improve graft survival in patients with established CAN in clinical trials. Thromboxane inhibitors and prostacyclin infusions have been shown to prevent the development of CAN in rats[15]. Some investigators have suggested that platelet aggregation and release of smooth muscle cell mitogens in response to damaged endothelium is an important step in the development of CAN[28], a theory supported by the prolongation of renal allograft survival by the antiplatelet agent Satrigel (E5510, Eisai, Tokyo, Japan)[29]. A similar pathogenesis has also been suggested for the development of intimal hyperplasia in autologous vein grafts or following balloon angioplasty, but aspirin at low or moderate doses (70–150 mg) has no effect on neointima formation in these situations[30,31]. The effect of moderate-dose aspirin on CAN may therefore result from mechanisms other than its antiplatelet activity. Eicosanoids are important inflammatory mediators in the pathogenesis of ischaemia–reperfusion injury[15] and acute rejection[23], both major aetiological factors for CAN. In humans the administration of aspirin to machine-perfused kidneys reduces intrarenal eicosanoid production, attenuates the severity of subsequent ischaemia–reperfusion injury and improves graft survival at 3 years in comparison to that of controls[32,33]. Aspirin also attenuates the increased thromboxane production seen during rejection episodes in humans[23] and reduces the degree of microvascular thrombosis following acute renal allograft rejection in animals[34].

In summary, aspirin therapy following renal transplantation was associated with a reduced rate of early graft thrombosis but did not improve renal function or graft survival at 2 years. There was a trend towards lower chronic rejection rates with aspirin, although this may also reflect lower cyclosporin levels in the aspirin-treated group. Prospective randomized trials are needed, however, before aspirin can be recommended for routine use following renal transplantation.

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Original article

Perioperative blood transfusions, with or without allogeneic leucocytes, relate to survival, not to cancer recurrence

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Background: Perioperative blood transfusions are reported to be related to cancer recurrence and reduced survival. Different underlying mechanisms have been proposed, and allogeneic leucocytes in transfused blood have been suggested to contribute to this phenomenon.

Methods: Packed red cells without buffy coat (PC group) were compared with filtered, leucoreduced, red cells (LD group) in a randomized trial of 697 patients with colorectal carcinoma. Five-year survival and cancer recurrence rates were determined, with special emphasis on the location of recurrence.

Results: The intention-to-treat analysis showed a survival rate of 63·6 per cent in the PC group and 65·3 per cent in the LD group ($P = 0{\cdot}69$), with recurrence rates of 27·8 and 27·9 per cent respectively. The observational analysis showed a significant difference in survival between transfused and non-transfused patients (59·6 *versus* 72·9 per cent; $P < 0{\cdot}001$). The difference in cancer recurrence rate between transfused and non-transfused patients was not statistically significant (29·8 *versus* 24·3 per cent; $P = 0{\cdot}13$). Local recurrences were more frequent in transfused than non-transfused patients (11·9 *versus* 7·6 per cent; $P = 0{\cdot}09$).

Conclusion: Leucocyte depletion of perioperative transfused blood has no effect on long-term survival and/or cancer recurrence. Perioperative blood transfusions are associated with impaired survival, but not with cancer recurrence. The slight increase in local recurrence rate in transfused patients appears to be related to complicated, in particular rectal, surgery.

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Introduction

Allogeneic leucocyte transfusion has immunomodulatory effects, and a negative effect of perioperative transfusion on postoperative cancer immunosurveillance is presumed[1,2]. In a randomized trial it was hypothesized that patients transfused with leucocyte-depleted blood would have a better cancer prognosis than patients transfused with packed cells without buffy coat. The median 2-year survival data and the in-hospital complications of this multicentre clinical trial (Cancer Recurrence And Blood transfusion (CRAB) study) have been reported previously[3]. No difference in mortality or morbidity rates between the randomization arms was seen, while in both arms transfused patients did significantly worse than non-transfused patients. Since this report new concepts about a deleterious effect of transfusion have been proposed, possibly explaining the contradictory results of previous studies[4,5]. Busch *et al.*[4] suggested a non-causal relationship in which the (rectal) location of the tumour played an important role, whereas Heiss *et al.*[5] described micrometastases that were observed to expand in transfused patients leading to late clinical recurrence beyond 2 years. Micrometastases have been described in approximately 50 per cent of patients with colorectal cancer[6]. In this 5-year survival evaluation additional subgroup analyses have been performed, focusing on transfused patients and on the location of recurrent cancer, to reveal late recurrence derived from metastatic growth.

Patients and methods

The design of the study and patient intake have been described previously[3]. When patients entered the study, technicians from the transfusion laboratories consulted an independent centralized data management group (Comprehensive Cancer Centre West, Leiden). The data management group performed random allocation to either the packed cells (PC) or the leucocyte-depleted (LD) trial arm by means of separate randomization lists for each of the participating hospitals. The (erythrocyte) blood products were either packed cells without buffy coat (PC arm), containing a mean of $0{\cdot}8 \times 10^9$ leucocytes per unit, or filtered, leucocyte-depleted, red cell concentrates (LD arm), containing a mean of $0{\cdot}2 \times 10^6$ white blood cells per unit. Data concerning the status of the patient during follow-up were gathered in each hospital, according to a standardized oncological follow-up scheme, and were available for all patients.

Statistical analysis

Cancer prognosis was measured in terms of survival rate and disease-free interval/recurrence risk. For comparison of qualitative parameters the χ^2 test was used, and for comparison of quantitative parameters the t test or Mann–Whitney U test was applied. To analyse survival and recurrence risk, Kaplan–Meier curves were calculated and differences between survival curves were assessed with the log rank test. Comparison of results between the two trial arms was performed on several study populations: (1) all patients who underwent potentially curative resection ($n = 697$), (2) all transfused patients ($n = 446$), (3) all patients with rectal or rectosigmoid carcinoma ($n = 339$) and (4) all transfused patients with rectal or rectosigmoid carcinoma ($n = 243$) (*Fig. 1*).

Additionally, a multivariate analysis of risk factors was performed, and an observational analysis was done comparing the prognosis of transfused patients with that of non-transfused patients.

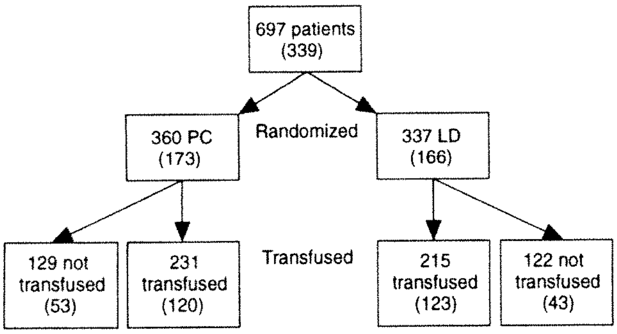


Fig. 1 Flow diagram showing patients included, randomized and transfused. Values in parentheses are numbers of patients with rectal or rectosigmoid carcinoma. PC, patients randomized to receive packed red cells without buffy coat; LD, patients randomized to receive filtered, leucocyte-depleted red cells

Results

Survival

At 5 years (median follow-up 66 months) the overall survival rate in this study population was 64·4 per cent. The intention-to-treat analysis showed a similar survival rate in the PC and LD trial arms (63·6 *versus* 65·3 per cent; $P = 0{\cdot}69$) (*Table 1*). The survival curves are shown in *Fig. 2*.

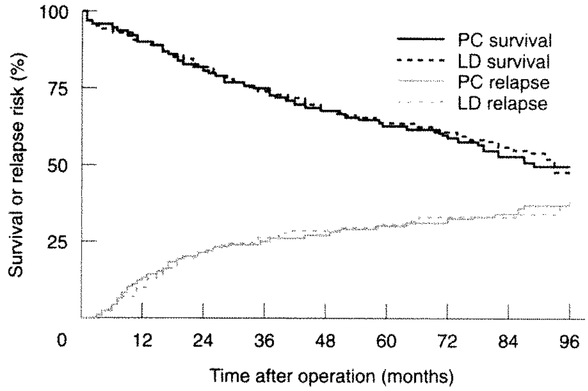
In the subgroup analyses small, non-significant, differences in survival were seen between the trial arms (*Fig. 3*, *Table 1*). The largest difference was found in the group of transfused patients with rectal or rectosigmoid carcinoma (51·7 and 60·2 per cent in PC and LD groups respectively; $P = 0{\cdot}20$).

In the observational analysis the non-transfused patients had a 5-year survival rate of 72·9 per cent compared with 59·6 per cent in the transfused patients ($P < 0{\cdot}001$) (*Table 2*).

Table 1 Survival and recurrence analyses at 5 years, according to intention to treat

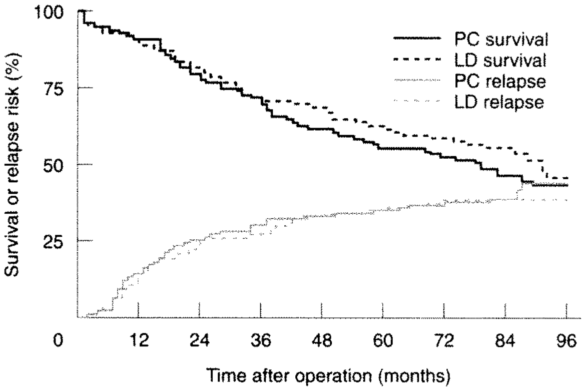
	Total	PC	LD	*P*
Total population				
No. at risk	697	360	337	
Survival (%)	64·4	63·6	65·3	0·69
Recurrence (%)	27·8	27·8	27·9	0·99
Local	10·3	11·1	9·5	
Regional	5·3	5·8	4·7	
Distant	18·5	18·3	18·7	
All transfused patients				
No. at risk	446	231	215	
Survival (%)	59·6	58·0	61·4	0·50
Recurrence (%)	29·8	31·2	28·4	0·54
Local	11·9	12·6	11·2	
Regional	6·1	6·9	5·1	
Distant	18·4	19·0	17·7	
Rectal/rectosigmoid carcinoma				
All patients				
No. at risk	339	173	166	
Survival (%)	59·3	56·1	62·7	0·23
Recurrence (%)	32·4	32·9	31·9	0·91
Local	13·9	15·0	12·7	
Regional	5·3	5·8	4·8	
Distant	20·4	19·7	21·1	
Transfused patients				
No. at risk	243	120	123	
Survival (%)	56·0	51·7	60·2	0·20
Recurrence (%)	35·4	37·5	33·3	0·51
Local	15·6	16·7	14·6	
Regional	6·6	6·7	6·5	
Distant	20·6	20·8	20·3	

Overall recurrence rate is less than the sum of the local, regional and distant recurrence rates since in some patients tumour recurrence occurred at more then one site. PC, packed cells; LD, leucocyte-depleted cells



No. at risk									
Survival	PC	360	323	288	263	238	214	143	68
	LD	337	302	276	242	225	205	149	69
Relapse	PC	360	297	260	239	222	202	134	63
	LD	337	284	247	219	200	187	131	61

Fig. 2 Percentage survival and percentage relapse during follow-up for the total population of 697 patients with colorectal carcinoma. Patients were randomized to receive packed red cells without buffy coat (PC) or filtered, leucocyte-depleted red cells (LD)



No. at risk									
Survival	PC	173	158	134	121	103	92	63	33
	LD	166	147	134	118	111	99	75	36
Relapse	PC	173	141	119	106	93	84	57	30
	LD	166	135	114	108	96	90	66	32

Fig. 3 Percentage survival and percentage relapse during follow-up for the subgroup of 339 patients with rectal or rectosigmoid carcinoma

Table 2 Survival and recurrence analyses at 5 years, according to transfusion status

	Non-transfused	Transfused	*P*
Total population			
No. at risk	251	446	
Survival (%)	72·9	59·6	< 0·001
Recurrence (%)	24·3	29·8	0·13
Local	7·6	11·9	0·09
Regional	4·0	6·1	0·29
Distant	18·7	18·4	0·92
Rectal/rectosigmoid carcinoma			
No. at risk	96	243	
Survival (%)	67·7	56·0	0·06
Recurrence (%)	25·0	35·4	0·09
Local	9·4	15·6	0·18
Regional	2·1	6·6	0·16
Distant	19·8	20·6	0·99

Recurrence

In the total population, recurrence rates were similar for the two trial arms (27·8 *versus* 27·9 per cent; $P = 0{\cdot}99$) (*Table 1*). Subgroup analysis revealed no difference in recurrence rate between the two transfusion protocols used. Even when recurrence was specified according to the site (11·1 *versus* 9·5 per cent local recurrence, 5·8 *versus* 4·7 per cent regional recurrence, and 18·3 *versus* 18·7 per cent distant recurrence for PC and LD arms respectively), none of these comparisons showed a significant difference between the two trial arms.

In the observational analysis, the cancer recurrence rates were 24·3 and 29·8 per cent in the non-transfused and transfused patients respectively ($P = 0{\cdot}13$). Distinguishing between sites of recurrence, local recurrences showed the biggest difference; the local recurrence rate was 7·6 per cent in non-transfused patients *versus* 11·9 per cent in transfused patients ($P = 0{\cdot}09$) (*Table 2*). In the subgroup of patients with rectal or rectosigmoid carcinoma the recurrence rate was 25 per cent in non-transfused patients and 35·4 per cent in transfused patients ($P = 0{\cdot}09$). Specified according to the site of recurrence, non-transfused and transfused patients with rectal or rectosigmoid carcinoma had local recurrence rates of 9·4 and 15·6 per cent, regional recurrence rates of 2·1 and 6·6 per cent, and distant recurrence rates of 19·8 and 20·6 per cent respectively (*Table 2*).

Risk factors

Potential risk factors for survival and colorectal cancer recurrence were first analysed univariately for their relation with survival and recurrence. The factors found to be significantly related to survival and/or recurrence were then entered into stepwise multivariate analyses. Most risk factors reported in the literature were also found to be related to survival and/or recurrence in this study. In the multivariate analyses, Dukes' stage and tumour invasion of blood vessels and lymph vessels were shown to have the strongest independent relation with both survival and

Table 3 Risk factors for survival and/or recurrence

	Univariate		Multivariate	
	Survival	Recurrence	Survival	Recurrence
Dukes' stage	< 0·0001	< 0·0001	< 0·0001	< 0·0001
Vessel invasion	< 0·0001	< 0·0001	< 0·0001	< 0·0001
Tumour size	< 0·0001	0·013	—†	—†
Grade of tumour differentiation	0·0001	0·0009	0·0035	n.s.
Tumour shape*	0·0018	0·0003	—†	—†
Postoperative infection	0·0002	0·018	n.s.	n.s.
Tumour extension to other organs	0·018	0·045	n.s.	n.s.
Age	< 0·0001	0·13	< 0·0001	n.s.
Perioperative transfusions	< 0·0001	0·19	0·0091	n.s.
Postoperative complications	0·0019	0·07	0·06	0·032
Continued use of anticoagulants	0·0052	0·24	n.s.	n.s.
Diabetes	0·0086	0·81	n.s.	n.s.
Chronic obstructive airway disease	0·021	0·38	0·027	n.s.
Tumour location	0·069	0·016	n.s.	0·019
Sex	0·13	0·22		
Pregnancies	0·13	0·32		
Blood loss	0·06	0·15		
Resection margin	0·64	0·65		
Plasma transfusions	0·05	0·39		
Trial arm	0·71	0·97		

*Scored as stalked, sessile, flat, circular or other. †Not included in multivariate analyses, because more than 10 per cent of data missing. n.s., Not statistically significant in multivariate analysis

recurrence (*Table 3*). The patient's age showed a strong independent relation with survival only, as did the differentiation grade of the tumour and perioperative transfusion. The location of the tumour was found to be related independently to recurrence, but not to survival.

Discussion

The CRAB study was designed to compare buffy coat-depleted red blood cells and filtered, leucocyte-depleted red blood cells for a possible deleterious role of allogeneic leucocytes on colorectal cancer recurrence. After surgery 697 patients without manifest metastatic disease were eligible for evaluation.

The intention-to-treat analyses showed no differences in survival or cancer recurrence between the two trial arms (*Fig. 2* and *Table 1*). In the transfused subgroups, slightly better 5-year survival was seen in patients receiving leucocyte-depleted transfusions, most markedly in the most heavily transfused subgroup of patients with rectal or rectosigmoid carcinoma. However, these differences failed to reach statistical significance (51·7 *versus* 60·2 per cent) (*Table 1*). The Kaplan–Meier curves of the two trial arms in the rectal or rectosigmoid carcinoma subgroup were almost continuously superimposed for the first 3 years (*Fig. 3*). It was only after 3 years that a small difference was seen between the two trial arms. In patients undergoing cardiac surgery a significant reduction in mortality rate with perioperative transfusion of leucocyte-depleted blood compared with transfusion of buffy coat-poor packed cells has been reported[7]. The difference in mortality rate was restricted to patients receiving four or more transfusions, and was seen within 60 days after cardiac surgery. The increased incidence of delayed deaths in patients with colorectal cancer after standard red blood cell transfusions cannot therefore be attributed to the same aetiology.

Differences between the participating hospitals concerning patient population, perioperative protocols and record-keeping were observed. However, the stratification of the randomization procedure per hospital resulted in a similar effect of these factors on both trial arms within each hospital and consequently prevented their influence on the results in the intention-to-treat analyses.

Several meta-analyses have described a significantly worse outcome in transfused patients compared with non-transfused patients[8–11]. These differences in survival were reproduced in the observational analysis of the present study. In the total population a significantly better survival rate for non-transfused patients than transfused patients was observed (72·9 *versus* 59·6 per cent; $P < 0{\cdot}001$) (*Table 2*). A similar difference (81 *versus* 69 per cent) was already present in previous analyses[3]. Therefore, if blood transfusion does have an effect on survival, it seems to have its major effect shortly after transfusion, and not years later. The ± 9 per cent lower survival rates in both the transfused and non-transfused patient groups of the recent analysis are the result of the longer follow-up available. Imbalances between the groups compared, showing a higher incidence of rectal carcinoma in the transfused group, and the better clinical status of patients not requiring blood transfusions, make it unjust to ascribe the differences found in the observational analysis solely to the transfusion status of the patients.

The cancer recurrence rate of 29·8 per cent in the transfused group was not significantly higher than the rate of 24·3 per cent in the non-transfused group ($P = 0{\cdot}13$). Distant metastases, becoming manifest after 2 years, are observed in up to 30–50 per cent of patients with gastrointestinal carcinoma[5,12]. Their growth seems to be influenced by variations in homing receptors on the tumour cells and alterations of adhesion molecules on endothelial cells[13,14]. Blood transfusion may influence the expression of these receptor molecules. However, the hypothesis put forward by Heiss and colleagues in relation to patients with gastric cancer[5], that blood transfusion may have a detrimental effect on recurrence in patients who already have micrometastases, could not be confirmed. In such a scenario a higher incidence of distant metastases in transfused patients should have been found, which was not the case in the present study population (18·4 *versus* 18·7 per cent; $P = 0{\cdot}92$) (*Table 2*). Local tumour recurrences may be the result of *a priori* unfavourable circumstances for removing the tumour completely. The present data supported the suggestion made by Busch *et al.*[4] that local circumstances leading to more transfusions will also lead to more local recurrences. Busch and colleagues observed a 20 per cent difference in local recurrence rate. The present study found a smaller difference in local recurrence rate between the transfused and non-transfused populations (11·9 *versus* 7·6 per cent; $P = 0{\cdot}09$) (*Table 2*)

Analyses of risk factors for survival and cancer recurrence are consistent with the hypothesis that transfusions do not influence recurrence but affect non-cancer death. Cancer stage and invasion of the tumour into lymph or blood vessels were the most important factors, influencing both survival and recurrence. Age, as might be expected, had a strong influence on survival, but not on recurrence. The same was true for the differentiation grade of the tumour and for perioperative blood transfusion. Perioperative transfusion correlated with survival in univariate and multivariate analyses, but not with cancer recurrence. Transfusion may have a detrimental effect on survival, but the observed association may also reflect the fact that transfusions are a marker for postoperative infectious complications, for more seriously ill patients and for other, possibly unknown, detrimental factors for survival.

Leucocyte depletion of transfused packed cells without buffy coat had no effect on the overall 5-year survival or on cancer recurrence. Perioperative blood transfusion, of both packed cells without buffy coat and leucocyte-depleted red blood cells, was correlated with shorter survival, but had no effect on cancer recurrence. The presumed negative effect on local recurrence of rectal carcinoma is probably due to the unfavourable surgical circumstances in this region and is not aggravated by allogeneic leucocytes in transfused blood. The occurrence of distant metastasis of colorectal carcinoma is not influenced by blood transfusion at all.

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Original article

Reduction of postoperative morbidity and mortality in patients with rectal cancer following the introduction of a colorectal unit

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Background: Surgery for rectal cancer is associated with high morbidity and mortality rates. The reason for this has been much debated. This population-based study reports the findings on postoperative morbidity and mortality after rectal cancer surgery following the introduction of a centralized colorectal unit in a county central hospital, supervised by a colorectal surgeon using the most recent techniques.

Methods: All consecutive patients with rectal cancer who underwent surgery at four county hospitals in the Västmanland county in Sweden during 1993–1996 ($n = 133$) were compared with patients who underwent surgery at the new colorectal unit in the county central hospital from 1996 to 1999 ($n = 144$).

Results: The number of operating surgeons was reduced from 26 to four. The postoperative mortality rate decreased from 8 to 1 per cent ($P = 0{\cdot}002$) and the total postoperative complication rate was reduced from 57 to 24 per cent ($P < 0{\cdot}001$). Surgical complications dropped from 37 to 11 per cent ($P < 0{\cdot}001$). The relaparotomy rate fell from 11 to 4 per cent ($P < 0{\cdot}05$). Postoperative stay in hospital was reduced from a median of 13 to 9 days ($P < 0{\cdot}001$).

Conclusion: The new organization, with centralized rectal cancer surgery using modern techniques, reduced postoperative mortality and overall morbidity rates to less than half.

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Introduction

Surgical complications after rectal cancer surgery have been reported frequently, in up to 30 per cent of patients. In papers in which all postoperative complications have been included, the total complication rate may exceed 50 per cent[1–3]. Numerous clinical and intraoperative risk factors have been proposed and studied. In recent years, the individual surgeon and the size and types of hospital have all been identified as potential independent risk factors[4,5].

Before 1996, rectal cancer surgery was performed at a central hospital and three other hospitals in a county in Sweden with a catchment area of 260 000 inhabitants. In January 1996, a colorectal surgeon trained at a university hospital was employed at the central county hospital. A colorectal unit was established and, since 1997, all rectal cancer surgery in the county was brought under the control of the central hospital mainly because of a decision made by the county politicians. New surgical procedures were introduced, including total mesorectal excision (TME)[6], pouch surgery and local excision with transanal endoscopic microsurgery (TEM)[7].

The aim of this study was to audit the introduction of a new organization, with a colorectal unit led by a colorectal surgeon using the most modern techniques, and a centralization of rectal cancer surgery in a well defined county with regard to changes in various operative variables, and postoperative morbidity and mortality.

Patients and methods

In the county of Västmanland, Sweden, 133 consecutive patients with rectal cancer had surgery at the district hospitals in Sala, Fagersta and Köping between January 1993 and December 1996, and at the central county hospital in Västerås between January 1993 and December 1995 (group 1). General surgeons with varying experience in colorectal surgery performed the operations, except for a few operations that were conducted by a locum colorectal surgeon during 1995. The medical records of these 133 patients were reviewed retrospectively. From January 1996 all rectal cancer surgery at the central county hospital in Västerås was performed or closely supervised by a newly employed colorectal surgeon and from January 1997 all rectal cancer surgery in the county was performed routinely at the central hospital. Between January 1996 and December 1999, 144 consecutive patients with rectal cancer were operated on within the new organization in Västerås (group 2) and the data were registered prospectively. Various operative and postoperative variables were compared between the two groups.

Definitions

Rectal cancer was defined as a tumour with the lowest tumour margin up to 15 cm from the anal verge. Postoperative complications and deaths were defined as those that occurred within 30 days of the primary operation. Surgical complications were bleeding, wound rupture, wound infection with pus, abscess, clinically non-healed anastomosis, fistula, stoma complications and urinary catheter at discharge. Infectious complications included urinary infection, pneumonia, sepsis and fever of unknown cause treated with antibiotics. Cardiovascular complications consisted of thromboembolism, infarction, dysrhythmia and cardiac decompensation or pulmonary oedema. The group 'miscellaneous' included compartment syndrome, nerve paraesthesia as a result of improper positioning on the operation table, liver or kidney disease, gastric stress ulceration, etc.

All patients received antithrombotic therapy (low molecular weight heparin) and antibiotic prophylaxis (usually cefuroxime 1500 mg and metronidazole 1500 mg administered intravenously at induction of anaesthesia). In both organizations a continuous epidural anaesthetic was offered to suitable patients.

Surgery

In group 1 only a few TME and colon pouch operations were performed and the resections were done using traditional procedures with generally blunt dissection. In group 2 low anterior resection with TME and reconstruction with a colonic pouch was the standard procedure. The dissection was largely performed with diathermy and the surgeon wore a headlight to enhance visual acuity. High ligature of the inferior mesenteric artery was performed and the splenic flexure taken down, ligating the inferior mesenteric vein at the lower border of the pancreas. After wash out of the stump, the anorectum was divided at the level of the levators or just below with a linear stapler. The descending or transverse colon with adequate arterial circulation was mostly used for constructing a short J pouch (5–6 cm) with a linear stapler. Tension-free anastomosis was completed with a circular stapler and the integrity was checked by palpation, inspection of the 'doughnuts' and air testing. Two suction drains were placed in the pelvis for 1–3 days. Abdominoperineal resections were performed with TME and as much as possible from the abdomen, avoiding synchronous combined excision.

In old and frail patients with suitable tumours, local resections, including TEM, were performed. For certain patients in whom an anastomosis was not suitable, a low resection with TME and division of anorectum was performed, followed by sigmoid colostomy (low Hartmann).

Statistical analysis

All analyses were performed using SPSS for Windows 9.0 (SPSS, Chicago, Illinois, USA). Differences between proportions were analysed with the χ^2 test. All other differences were examined using the Wilcoxon–Mann–Whitney test because distributions in the samples were not Gaussian. The level of statistical significance used was $P < 0\cdot05$.

Results

The clinical details of the patients and tumours are summarized in *Table 1*. There were no significant differences between the two groups, except in Dukes' staging; there was a greater proportion of Dukes' C tumours in group 2. About half of the patients in both groups received

Table 1 Clinical details of patients and tumours

	Group 1	Group 2
No. of patients	133	144
Age (years)*	70 (30–93)	69 (40–95)
Sex ratio (M : F)	76 : 57	84 : 60
Preoperative comorbid conditions†	63 (47)	66 (46)
Fixed cancer†	14 (11)	15 (10)
Tumour height (cm)*	8·6 (0–15)	7·7 (2–15)
Preoperative radiotherapy†	79 (59)	70 (49)
Dukes' stage†‡		
A	32 (25)	37 (27)
B	47 (36)	33 (24)
C	23 (18)	45 (32)
'D'	27 (21)	24 (17)

*Values are mean (range); †values in parentheses are percentages. ‡Dukes' stage could not be assessed in four patients in group 1 and five in group 2

Table 2 Surgical procedures

	Group 1 ($n = 133$)	Group 2 ($n = 144$)
Explorative laparotomy	1 (1)	0 (0)
Sigmoid colostomy only	15 (11)	13 (9)
Local excision	1 (1)	7 (5)
Transanal resection	1 (1)	3 (2)
Anterior resection	56 (42)	5 (3)
Low anterior resection + colonic pouch	5 (4)	79 (55)
Proctectomy + ileal pouch	0 (0)	1 (1)
Hartmann's procedure	3 (2)	7 (5)
Abdominoperineal resection	51 (38)	26 (18)
Proctocolectomy + ileostomy	0 (0)	3 (2)

Values in parentheses are percentages

Table 3 Operative details

	Group 1 ($n = 133$)	Group 2 ($n = 144$)
Emergency operation*	4 (3)	2 (1)
Laparoscopic procedure*	0 (0)	4 (3)
Operation time (min)†	182 (30–445)	247 (20–540)**
Blood loss (ml)†	1422 (0–13 500)	791 (0–3400)**
Blood transfusion (ml)†‡	1170 (0–12 000)	477 (0–2800)**
Intraoperative complications*§	17 (13)	8 (6)
Anastomosis-related problems*¶	16 of 61 (26)	1 of 85 (1)

*Values in parentheses are percentages; †values are mean (range). ‡Blood transfusions included all transfusions given during hospital stay. §Substantial bleeding, organ injury, faecal contamination or cardiac problems. ¶Incomplete doughnut, air leakage, palpable defect or resuturing/restapling. **$P < 0{\cdot}001$ *versus* group 1. Mann–Whitney U test

Table 4 Postoperative complications

	Group 1 ($n = 133$)	Group 2 ($n = 144$)
Infectious	26 (20)	13 (9)*
Cardiovascular	14 (11)	5 (3)*
Miscellaneous	7 (5)	8 (6)
Surgical	49 (37)	16 (11)†
Wound infection	14 (11)	8 (6)
Wound dehiscence	3 (2)	0 (0)
Stoma complications	6 (5)	1 (1)
Postoperative bleeding	7 (5)	1 (1)
Postoperative intestinal obstruction	2 (2)	0 (0)
Anastomotic dehiscence	7 (5)	3 (2)
Colonic ischaemia	0 (0)	1 (1)
Fistula	2 (2)	1 (1)
Deep abscess	3 (2)	2 (1)
Urinary catheter *in situ* at discharge	14 (11)	2 (1)

Values in parentheses are percentages. Some patients had more than one complication. *$P < 0{\cdot}05$, †$P < 0{\cdot}001$ *versus* group 1. χ^2 test

preoperative radiotherapy of 25 Gy in five fractions over 5 consecutive days. Stratification according to the American Society of Anesthesiologists (ASA) classification showed no difference between the groups (data not shown). No patient received preoperative chemotherapy.

In group 1, 26 surgeons performed a mean of 5 (range 1–18) operations per surgeon during a 3–4-year period, depending on hospital. In group 2, two surgeons operated on 140 (97 per cent) of the 144 patients. The remaining four patients required palliative non-resectional procedures, which were performed by two other surgeons. The surgical procedures are listed in *Table 2*. Based on the histology reports, complete excision was achieved in 93 (85 per cent) of 109 resections in group 1; in seven (three Dukes' 'D') resections, no assessment of radical excision was performed. In group 2, TME resections were performed in 116 (81 per cent) of the 144 patients and complete excision was noted in 120 (94 per cent) of the 128 patients who had a resection. Other organs were resected in 20 patients in group 1 compared with 25 patients in group 2, although *en bloc* resections constituted five of 20 cases in group 1 compared with 15 of 25 cases in group 2. The proportion of anterior resections compared with abdominoperineal resections increased from 54 per cent in group 1 to 77 per cent in group 2. Only one abdominoperineal resection was performed for a tumour above 5 cm from anal verge (6 cm) in group 2 compared with 23 such resections in group 1.

Operative details are given in *Table 3*. The laparoscopic procedures performed in group 2 were stoma operations for non-resectable tumours.

Eleven patients (8 per cent) died after operation in group 1 compared with one patient (1 per cent) in group 2 ($P = 0{\cdot}002$). In total, postoperative complications occurred in 76 patients (57 per cent) in group 1 and in 34 patients (24 per cent) in group 2 ($P < 0{\cdot}001$). No significant differences were noted in total complication rate between the four different hospitals in group 1. The types of complication are given in *Table 4*. Under 'miscellaneous', two patients in group 2 developed compartment syndromes during the first few months. Reoperation was necessary in 23 patients (17 per cent) in group 1 and in ten patients (7 per cent) in group 2 ($P = 0{\cdot}008$). Laparotomy, as a consequence of complications, was performed in 14 patients (11 per cent) in group 1 compared with six patients (4 per cent) in group 2 ($P = 0{\cdot}04$). Complications after anterior resection and abdominoperineal resection are listed in *Table 5*. A diverting stoma was used in selected cases in both groups: ten patients (16 per cent) in group 1 and in 28 patients (33 per cent) in group 2. Postoperative stay in hospital was reduced from a median of 13 (range 1–125) days in group 1 to 9 (range 0–59) days in group 2 ($P < 0{\cdot}001$).

Table 5 Complications after anterior resection and abdominoperineal resection

	Anterior resection		Abdominoperineal resection	
	Group 1 (*n*=61)	Group 2 (*n*=85)	Group 1 (*n*=51)	Group 2 (*n*=26)
Total postoperative complications	33 (54)	18 (21)†	34 (67)	10 (38)*
Surgical complications	18 (30)	9 (11)†	25 (49)	5 (19)*
Anastomosis-related complications	8 (13)	5 (6)	—	—
Reoperation	7 (11)	8 (9)	13 (25)	0 (0)†
Relaparotomy	6 (10)	5 (6)	5 (10)	0 (0)
Death	1 (2)	0 (0)	3 (6)	0 (0)

Values in parentheses are percentages. $^*P<0{\cdot}05$, $^\dagger P<0{\cdot}001$ *versus* group 1. χ^2 test

Discussion

This study has demonstrated that rectal cancer surgery concentrated in a central unit, led by a trained colorectal surgeon, is associated with significantly reduced postoperative complication and mortality rates. More patients had sphincter-saving procedures, fewer reoperations and relaparotomies were necessary, and there was a shorter postoperative hospital stay. The operation time was prolonged in the new organization but there was a reduction in peroperative bleeding and in the number of blood transfusions. In two patients, however, the long operating procedures caused compartment syndromes during the first months in the new organization and a change in protocol was made such that the legs were placed in the lithotomy position only during reconstruction or during the perineal phase of dissection in abdominoperineal resection.

Previous studies have reported that the complication and anastomotic leakage rates increase the closer the anastomosis is to the anus[8,9]. Other authors have suggested that TME surgery is associated with a high morbidity rate[1]. In a recent study, anastomotic leakage was reported to have occurred in six of 37 patients, with one death[10]. In the present study a significant reduction in total morbidity was observed, including surgical postoperative complications with no death. Anastomosis-related healing problems were reduced from a somewhat high level of 13 per cent in group 1, in which the traditional anterior resection was used, to 6 per cent in group 2, in which TME resection and reconstruction with a colonic pouch was used. Contrary to a recent study[11], no increase in leakage was found after the introduction of TME. These positive results may be related to the fact that the colorectal surgeon was experienced in TME surgery, having used the procedure over a period of several years.

In this study retrospective data from the old organization (group 1) were compared with prospective data from the new organization (group 2). Although not optimal, this method probably underestimates the complication rate in the old organization because it is probable that some complications were never registered in the case records. The two organizations were comparable regarding clinical details of patients, including preoperative risk assessment of patients by ASA grade. All operations for rectal cancer performed in the county were included without applying any selection criteria; however, some referrals of difficult cases in the old organization were made to university hospitals for further treatment. The time intervals compared were close and there were no differences in perioperative and anaesthetic conditions.

Past efforts to explain short-term outcomes after rectal cancer surgery have focused on different risk factors, such as presurgical and intraoperative variables, but few studies have concentrated on the surgeon or organization as determining factors. Studies from the UK have shown that the outcome after colorectal surgery varies considerably among surgeons in terms of postoperative morbidity and mortality. It has been suggested that colorectal cancer surgery should be undertaken by surgeons with a special interest in colorectal surgery[4,5]. On the other hand, some studies have failed to show any differences in postoperative mortality rate for operations performed by general surgeons[12,13]. A recent retrospective study found acceptable results after colorectal cancer surgery performed by non-specialist surgeons in a district hospital; however, only 48 of the 267 cases concerned were rectal or rectosigmoid cancers and they were not analysed separately[14]. A Swedish multicentre study found that postoperative surgical complications and death were not significantly related to the experience of the surgeon, the mean annual number of rectal cancer operations performed by the surgeon or the type of hospital[15]. In another study, a training record in colorectal surgery and case volume had no significant impact on postoperative morbidity or mortality rates[16]. In these studies, however, the case volume per surgeon per year was very low compared with that in the new organization in the present study.

In conclusion, a significant reduction in postoperative morbidity and mortality rates can be achieved after rectal cancer surgery in a well defined demographic setting by introducing a centralized colorectal unit directed by a trained colorectal surgeon using the latest surgical techniques.

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Original article

One-year survey of carcinoma of the oesophagus and stomach in Wales

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Background: The aim of the study was to identify all patients who presented with oesophagogastric malignancy within a single National Health Service region (Wales) over 1 year, and to follow the cohort for 5 years. Management and outcome were analysed to identify current practice and draft guidelines for Wales.

Methods: Patients were identified from hospital records. Details were recorded in structured format for analysis.

Results: Analysable data were obtained for 910 of 916 patients. The overall incidence was 31·4 per 100 000 population. Treatment was by resection 298 (33 per cent), palliation 397 (44 per cent) or no treatment 215 (24 per cent). The 30-day mortality rate was 12 per cent and the in-hospital mortality rate was 13 per cent. Some 226 patients (25 per cent) were alive at 2 years. Resection conferred a significant survival advantage over palliation ($P < 0{\cdot}001$) and no treatment. Anastomotic leakage occurred in 16 patients (5 per cent), of whom eight died in hospital. 'Open and close' operations were common (23 per cent), laparoscopy was infrequent (16 per cent), and many surgeons undertook small caseloads. Operating on fewer than six patients per year increased the mortality rate after partial gastrectomy ($P < 0{\cdot}05$) and was associated with a trend to a higher mortality rate after mediastinal and cardia surgery. Operating on more than 70 per cent of patients seen resulted in a significantly higher mortality rate ($P < 0{\cdot}01$) irrespective of case volume.

Conclusion: Tumour resection conferred a survival advantage. Wider use of laparoscopy is advocated. Improved selection for surgery should result in a lower mortality rate.

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Introduction

Carcinoma of the oesophagus, cardia and stomach is the fourth most common malignancy in England and Wales. The total number of patients affected fell from 16 259 registered cases in 1982 to 15 816 in 1992[1]. There appears to have been a slight increase in the incidence of oesophageal carcinoma over this 10-year period from 1982 to 1992 (males 9·8–13·8 per 100 000 population, females 7·3–9·0 per 100 000) and a decrease in the incidence of gastric carcinoma (males 29·8–24·9, females 19·0–14·7 per 100 000). While this may be due partly to a reclassification of cardia tumours as lower-third oesophageal tumours, it is generally accepted that there has been a decrease in the frequency of gastric body and antral carcinoma, and that tumours of the cardia and lower third of the oesophagus are now more prevalent[2,3].

In general, the results of treatment from this group of tumours are poor[4,5]. The technical expertise and team support that are found in specialist centres allow better results when compared with the overall results from non-specialist centres[6–13]. The stage of disease has a profound effect on outcome[12,14,15]. There is also general evidence to suggest that case volume per surgeon (or unit) is a factor in the outcome of treatment of malignant disease[9,16,17].

A population-based audit of colorectal carcinoma was carried out in Wales in 1993 to document practice in a National Health Service (NHS) region[18]. It was decided to audit and survey consecutively all cases of oesophageal and gastric malignancy in Wales (population of 2 921 100 in

1996) over a 12-month period. This cohort of patients will be followed for 5 years. The aim was to identify current practice and to provide a database from which guidelines for the future management of patients with oesophagogastric malignancy in Wales could be formulated.

Patients and methods

All hospital consultants in Wales involved in the management of patients with carcinoma of the oesophagus or stomach agreed to have their practice audited. All cases between September 1995 and August 1996 were identified from hospital records. Data were gathered from case notes, pathology laboratory records, hospital coding departments and operation registers in each hospital by three experienced research data collectors employed by the All Wales Audit and Research (AWARe) Unit in Cardiff. The data collectors were first employed in the All Wales Colorectal Study. They were audited for accuracy at that time, and demonstrated their ability to ensure that no cases were missed. They were found to be more accurate than the hospital coding departments[18]. In this study patients were tracked out of Wales, if referred out for treatment, and those treated in the private sector were also included. The data were coded confidentially, entered into a database by means of optical scanning, and data analysis was carried out with the Statistical Package for Social Sciences (SPSS) version 8.0 (SPSS, Chicago, Illinois, USA). Statistical analysis was performed with the χ^2 test, with Yates' correction for small numbers where appropriate.

The sites of tumours in the foregut were designated within each third of the oesophagus and the cardia (defined as the gastro-oesophageal junction). Gastric tumours were defined as fundic, body or antral. It is conceded that some blurring of definition is inevitable with tumours arising around the cardia. In this series, the site of the tumour was based on preoperative investigations. In the analysis of data, morbidity and mortality were assessed according to the type of operation. Analysis was carried out by separating the operations into those performed in the mediastinum, at the cardia and in the abdomen.

Results

There were 916 patients identified; of these, 910 case notes were available for analysis. This gave an overall incidence of 31·4 per 100 000 population. The age distribution of the patients is shown in *Fig. 1*; 61 per cent were over the age of 70 years.

Seventy consultant surgeons saw 640 (70 per cent) of the 910 patients. Only 21 surgeons (30 per cent) saw more than ten patients during the year. Thirty-two surgeons saw three or fewer patients in the year. Sixty-three of the 70 surgeons performed operations on the patients included in this audit. There was wide variation in the numbers of operations performed by individual surgeons, as shown in *Figs 2* and *3*. Forty-three surgeons performed up to five operations and four operated on more than 20 patients in the year. Nearly one-quarter of the patients (215) had no active therapy; the majority of these, 143 (67 per cent), were deemed to be unfit for surgical treatment. There were 298 patients (33 per cent) who underwent resection. The remaining 397 patients had palliative treatment with a variety of interventions such as dilatation, stenting, chemotherapy or radiotherapy. This group included patients who underwent 'open and close' laparotomy or thoracotomy.

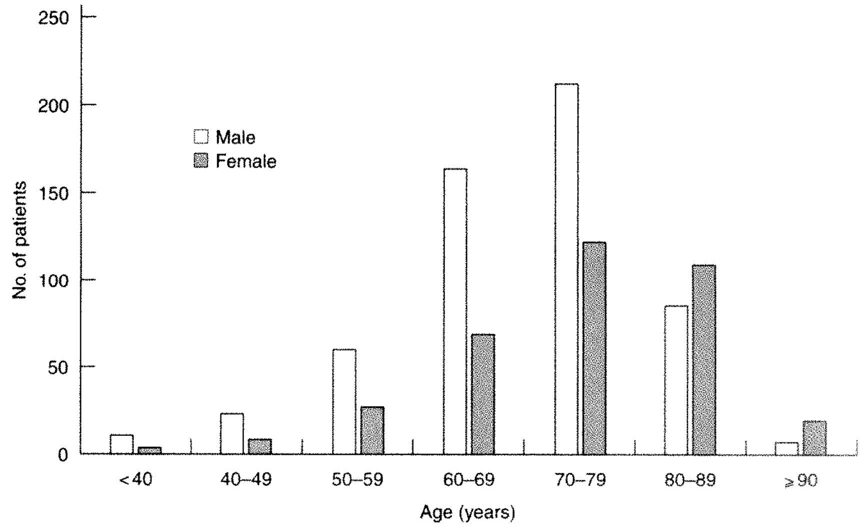

Fig. 1 Age distribution of patients

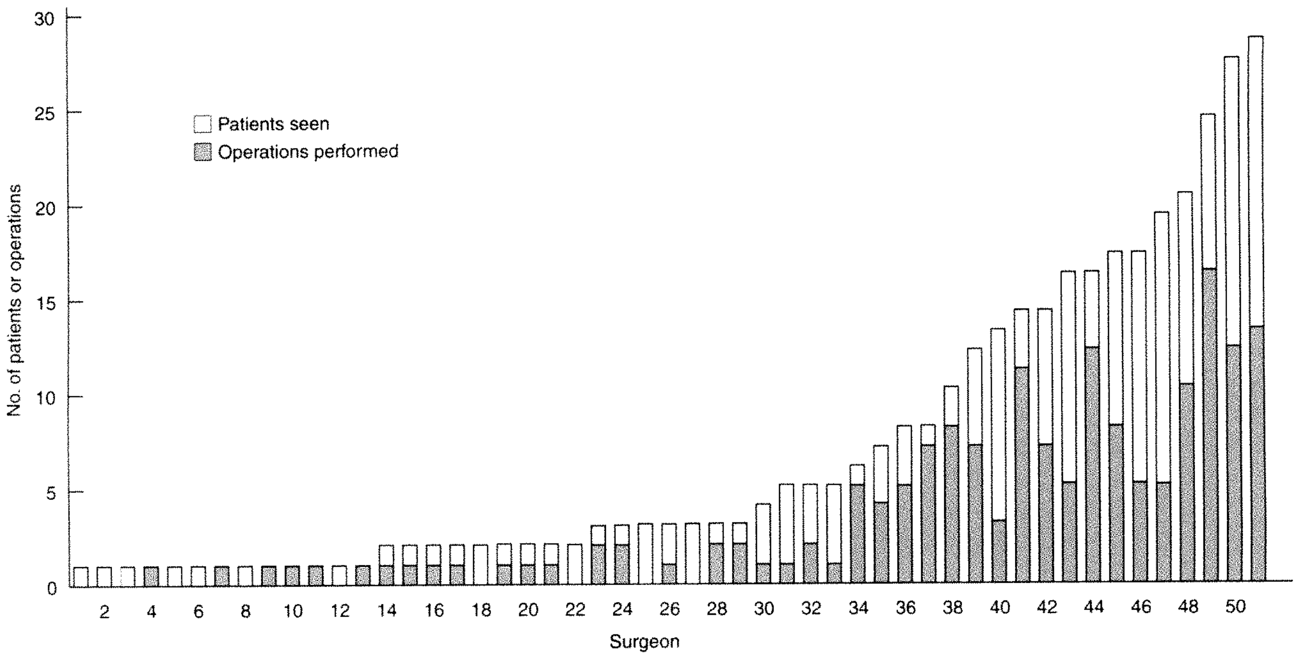

Fig. 2 Number of patients with oesophageal or cardia carcinoma seen and operations performed per surgeon

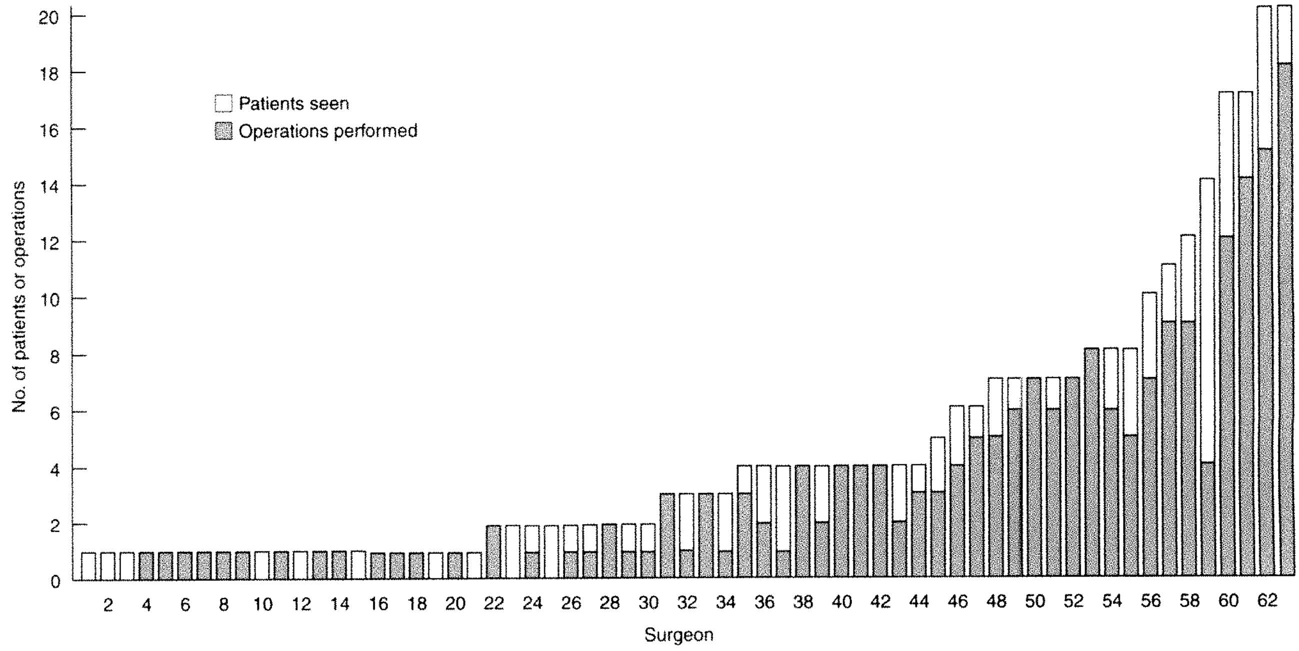

Fig. 3 Number of patients with stomach carcinoma seen and operations performed per surgeon

Site, presentation and investigation of carcinoma

Weight loss was the most common presenting symptom, present in 63 per cent of patients with oesophageal tumours, in 66 per cent of those with cardia tumours and in 59 per cent with gastric tumours. This confirmed the late presentation documented by others[19,20]. Oesophagogastroduodenoscopy (OGD) and biopsy were carried out on almost all patients. Computed tomography was performed in 70 per cent of patients with oesophageal tumours and in 59 per cent of those with cardia or gastric tumours. Barium studies were performed in 42 per cent of patients with oesophageal tumours and in 31 per cent of those with cardia or gastric tumours. Ultrasonography was used in 64 and 58 per cent of patients respectively. Magnetic resonance imaging was performed in one patient, and bronchoscopy in five. Laparoscopy was carried out in 5 per cent of patients with oesophageal tumours and in only 16 per cent of those with cardia or gastric tumours. The distribution of tumour sites is shown in *Fig. 4*. Clinical details of patients with tumours at the three main sites are given in *Table 1*.

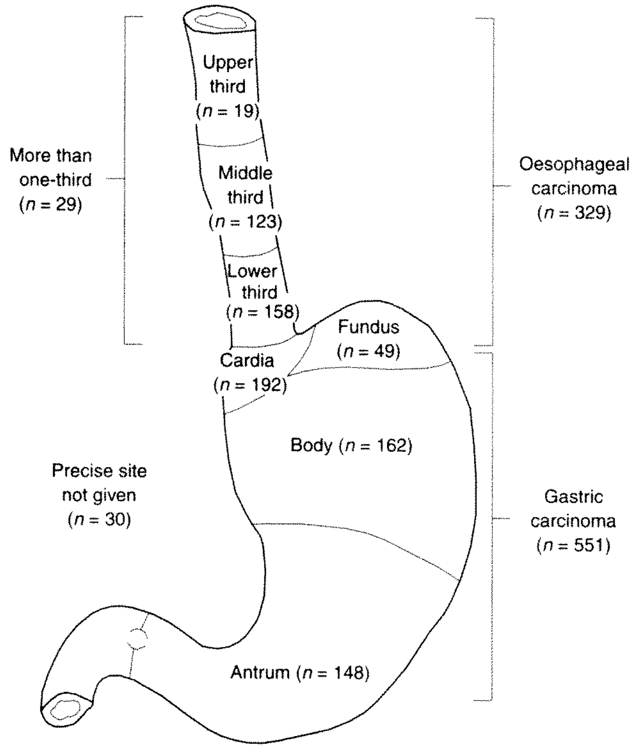

Fig. 4 The sites of tumours identified by investigation

Type of operation

Three hundred and eighty-nine patients (43 per cent) had an operation. Of the patients who underwent operation, 298 (77 per cent) had a resection. Eighty-nine patients (23 per cent) had an 'open and close' laparotomy or thoracotomy. The specific procedure was not recorded for two patients. Of the patients who underwent definitive surgical procedures, 111 had mediastinal surgery, 139 had cardia surgery and 139 had an abdominal partial gastrectomy. *Table 2* details the procedures and 30-day mortality associated with each type of operation.

Anastomotic leakage

Of the 298 patients who underwent anastomosis, 16 (5 per cent) developed an anastomotic leak that was clinically or radiologically evident; eight of these 16 patients died. The procedures associated with anastomotic failure are given in

Table 1 Clinical details of patients with tumours at the three main sites

	Oesophagus	Cardia	Stomach
No. of patients	337	192	381
Sex ratio (M : F)	1·4 : 1	1·6 : 1	1·7 : 1
Mean age (years)	69·9	71·7	72·9
(range)	(37–93)	(33–93)	(21–95)
Surgery performed	90 (27)	86 (45)	213 (55)
Resectional surgery performed	72 (21)	57 (30)	169 (44)
Tumour spread noted	99 (29)	69 (36)	139 (36)
Local	29	25	36
Distant	68	43	98

Values in parentheses are percentages

Table 2 Surgical procedures and 30-day mortality rates

	Mediastinal surgery		Cardia surgery		Abdominal surgery	
Procedure	*n*	Deaths*	*n*	Deaths*	*n*	Deaths*
Transhiatal oesophagectomy	10	2	—	—	—	—
McKeown (three stages)	17	1	—	—	—	—
Ivor Lewis (two stages)	62	5	—	—	—	—
Left thoracoabdominal	4	0	—	—	—	—
Total gastrectomy	—	—	98	14	—	—
Proximal gastrectomy	—	—	12	4	—	—
Distal gastrectomy	—	—	—	—	95	4
'Open and close'†	16	2	29	4	44	9
Procedure not specified	2	0	—	—	—	—
Total	111	10 (9)	139	22 (16)	139	13 (9)

Values in parentheses are percentages. *Thirty-day mortality rate. †Thoracotomy and laparotomy only

Table 3 Procedures complicated by anastomotic leak

Type of excision	Leak rate
Ivor Lewis	6 of 62 (10)
McKeown (three stages)	1 of 17 (6)
Total gastrectomy	7 of 98 (7)
Proximal gastrectomy	2 of 12 (17)

Values in parentheses are percentages

Table 4 Mortality rate by proportion of patients undergoing operation

	Proportion of patients treated surgically	
	> 70%	< 70%
Total no. of patients	134	160
No. of deaths	24	8
30-day mortality rate (%)	18	5

$\chi^2 = 10{\cdot}0$, 1 d.f., $P < 0{\cdot}01$

Table 3. There was no correlation between anastomotic failure and surgical case volume.

Surgery, case selection and case volume

The surgeons who undertook resection, including those who were referred ten or more patients, appeared to fall into two groups, each with an apparently different treatment philosophy. There was an obvious, visible and wide separation in the proportion of patients being treated with surgery. In one group, the operative rate was more than 70 per cent with a 30-day mortality rate of 18 per cent. In the other group the operative rate was less than 70 per cent and was associated with a 30-day mortality rate of 5 per cent ($P < 0{\cdot}01$) (*Table 4*). Patient comorbidity was equally spread across the different hospitals and across surgeons with no particular bias either in favour of, or against, surgeons with a high proportion of operated cases.

Overall the 30-day mortality rate was 12 per cent and the in-hospital mortality rate was 13 per cent. Investigation into the outcomes related to caseload by surgeon is shown in *Table 5*. The results show a difference in favour of a greater caseload, but this was statistically significant ($P < 0{\cdot}05$) only in the case of abdominal partial gastrectomy.

Pathological data and survival

Histopathological details of the resected specimens and their relationship with survival are given in *Table 6*. There was no histology record in 75 patients (8 per cent). Overall, 306 patients (34 per cent) were alive at 1 year, and 226 (25 per cent) at 2 years. At 2 years patients who had undergone surgical resection had a considerable survival advantage (43 per cent) ($\chi^2 = 327{\cdot}3$, 1 d.f., $P < 0{\cdot}001$) over those who had received palliative treatment (17 per cent), who did not differ from those having no treatment (14 per cent). Patients with adenocarcinoma who were lymph node negative had a considerable survival advantage over those with lymph node positive disease ($\chi^2 = 15{\cdot}1$, 1 d.f., $P < 0{\cdot}001$).

Table 5 Deaths in hospital by case volume and operation type

	Total no. of operations	Deaths in hospital
Caseload < 6		
Mediastinal surgery	18	4 (22)
Cardia surgery	23	4 (17)
Abdominal surgery	29	3 (10)*
Open and close	31	6 (19)
Total	101	17 (17)
Caseload ≥ 6		
Mediastinal surgery	75	11 (15)
Cardia surgery	87	15 (17)
Abdominal surgery	66	1 (2)*
Open and close	60	8 (13)
Total	288	35 (12)

Values in parentheses are percentages. *χ^2 with Yates' correction = 3·89, 1 d.f., $P < 0{\cdot}05$

Discussion

This study is one of the few comprehensive studies of gastro-oesophageal malignancy in a single NHS region in the UK. The data collection was independent and impartial. The use of data collectors may be questioned, but these three collectors have been shown[18] to be reliable and accurate. Many previous papers have been retrospective reviews[6,9,12,19] or prospective audits of surgery for gastro-oesophageal malignancy emanating from specialist centres[8,10,13,14,20]. These studies therefore tend to reflect treatment in patients already selected for interventional therapy, and produce results that may not reflect the population as a whole. In Wales, only one-third of patients were suitable for resection. This is similar to the findings of another review from the UK, which may be compared with the present survey[19]. This demonstrated that 485 patients, 35 per cent of the total, underwent resection; 200 of these operations were considered potentially curative. The 5-year survival rate of patients who underwent resection was 22 per cent. The authors believed that surgery and results such as their own might remain a standard against which combined modality therapy could be compared. It will also provide a useful comparator when this study has completed 5 years of follow-up.

Table 6 Survival by histology and lymph node status of patients undergoing resection

Histology and lymph node status	Mediastinal resection No. of patients	Mediastinal resection Survival rate (%) 1 year	Mediastinal resection Survival rate (%) 2 years	Cardia and abdominal resection No. of patients	Cardia and abdominal resection Survival rate (%) 1 year	Cardia and abdominal resection Survival rate (%) 2 years
Adenocarcinoma						
Node positive	34 (37)	47	24	93 (45)	57	37
Node negative	15 (16)	73	60	68 (33)	71	61
Node not stated	13 (14)	62	46	20 (10)	40	25
Squamous carcinoma						
Node positive	8 (9)	75	50			
Node negative	11 (12)	82	82			
Node not stated	2 (2)	50	50	3 (1)	33	33
Other histology	5 (5)	20	20	8 (4)	40	40
Missing data	5 (5)	60	40	13 (6)	62	46
Total	93 (100)	58	42	205 (100)	59	43

Values in parentheses are percentages

The definition of tumours around the cardia remains a significant problem. A recent definitive classification has been published[21,22], but this is not yet universally accepted. The interpretation and description of tumour type is subjective. A lower-third oesophageal tumour to one observer may be recorded as a cardia tumour by another[22]. In upper gastrointestinal surgery, the main differences in outcome hinge less on the description of the tumour than on the type of operation carried out[7,8,13,15,20,23,24], the histology, and the stage and grade of the tumour[11–13,25,26]. In view of that, the analysis in this study focused on the type of operation rather than the tumour description. The additional technical awkwardness of access to the cardia is reflected in the higher mortality rate in these patients.

In 1980 Earlam and Cunha Melo[6] reviewed 83 783 patients reported in 122 publications to examine the surgical results of the treatment of squamous carcinoma of the oesophagus. In comparison, this survey has demonstrated the operative mortality rate for oesophageal squamous cell carcinoma to be less than half that reported by Earlam and Cunha Melo[6] and the 1-year survival rate to be more than three times greater. This survival benefit continues at least until the second year.

One of the obvious areas for improvement highlighted by this study is the high 'open and close' rate of 23 per cent, which seems unacceptable. The use of diagnostic laparoscopy in Wales was not well developed in 1996. Better preoperative case selection can be achieved by abdominal laparoscopy[27–29] and endoscopic ultrasonography[30,31].

The Welsh study has shown that a large number of surgeons was involved in the management of these patients, some managing only one or two cases per year. This may reflect practice in some other parts of the UK. Both Miller *et al.*[32] and Matthews *et al.*[16] studied the caseload per surgeon and considered that surgeons who performed six or more oesophageal resections per year obtained better results, in terms of improved perioperative mortality rate, than those who operated on fewer than six patients per year. Data from the present survey have not shown a strong volume effect with oesophageal or cardia surgery, which does not match previous studies[16,32]. There was, however, a significant difference favouring a higher case volume in abdominal gastric surgery. This does concur with the findings of McCulloch[9], who suggested that operations for gastric carcinoma are best carried out by surgeons with a special interest. The more interesting aspect is the difference in philosophy in surgical approach, which was obvious in the raw data, and which appears to be important in terms of postoperative mortality. The arguments for an aggressive surgical approach are to provide a better quality of palliation than stenting or laser recanalization of the tumour. The arguments against are to try to avoid untimely death by operation in the poorly selected patient. Patient selection is important in this disease[33] as patients usually have significant comorbidity and relatively advanced disease[11,25,26,34]. Traditionally, anastomotic failure has been the most common cause of death after oesophagectomy. In the present series the leak rate was 5 per cent, but only eight of the 16 affected patients died. This indicates that other factors are important in postoperative mortality. The significant difference in survival when more than 70 per cent of patients were treated surgically compared with less than 70 per cent, in conjunction with a low overall anastomotic leak rate, strongly suggests that case selection is a major factor.

Case selection protocols[33] can be developed to bring together all the strands that are important in the management of patients with upper gastrointestinal cancer. Key messages from a recent National Confidential Enquiry into Perioperative Deaths report[35] emphasized the need for a multidisciplinary team approach, with at least two surgeons expressing a major subspecialty interest in the management of these malignancies. It is concluded that patients suitable for resection have a survival advantage, but case selection is highly important in realizing that advantage. Wider use of laparoscopy and endoscopic ultrasonography is advocated to improve case selection. Wales should move towards having fewer surgeons, with multidisciplinary teams in place, to treat patients with upper gastrointestinal malignancy.

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Original article

Reduction of experimental adhesion formation by inhibition of plasminogen activator inhibitor type 1

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Background: Adhesion formation is a common cause of complications following surgery. A reduction in peritoneal fibrinolytic capacity during operation is a key mechanism in the early formation of adhesions. An increase in the main inhibitor of fibrinolysis, plasminogen activator inhibitor type 1 (PAI-1), is a major factor in the loss of fibrinolytic activity. The aim of this study was to investigate if inhibition of PAI-1 could reduce the formation of adhesions after surgery.

Methods: Mice ($n = 53$) were subjected to a standard surgical procedure in order to induce adhesion formation to the abdominal side wall. At the conclusion of the operation, fragments for antigen binding of polyclonal rabbit antibody against PAI-1 (PRAP-1) were injected intraperitoneally, at two different concentrations. Control animals received an equal volume of the vehicle (saline). One week after operation adhesion formation was quantified.

Results: Both doses of PRAP-1 significantly reduced adhesion formation compared with the saline control ($P = 0{\cdot}003$ and $P = 0{\cdot}002$). There were no signs of bleeding in the postoperative period or at reoperation.

Conclusion: The present observations lend further support to the hypothesis of a pivotal role of fibrinolysis in the early formation of adhesions, and open up new possibilities for adhesion reduction by inhibiting PAI-1.

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Introduction

Adhesion formation is an almost inevitable result of abdominal surgery, found in more than 90 per cent of patients who have undergone one or more abdominal operations[1]. Adhesions are the commonest cause of small bowel obstruction[2] and can lead to infertility in women[3]. The risk of inadvertent enterotomy is as high as 19 per cent in operations on patients who have previously had surgery[4]. This translates clinically into adhesion-related readmissions in a substantial proportion of patients[5].

The formation of adhesions after surgery seems to be determined by the balance of fibrin deposition and degradation in an early phase of peritoneal tissue repair. When two damaged peritoneal surfaces come into apposition, fibrinous adhesions form between the surfaces. In the presence of sufficient fibrinolytic activity there is a resolution of the fibrin strands. However, if there is insufficient fibrinolytic activity, organization of the fibrin matrix and cellular and vascular ingrowth ensues, leading to fibrous bands between the structures[6].

Support for the role of local fibrinolytic capacity derives from animal experiments and observations in humans. Experimentally, decreased fibrinolytic activity correlated with adhesion formation in animals[7]. In humans, observations during operation revealed that surgery elicited a rapid decline in peritoneal fibrin degradation capacity. This decline was caused partly by a reduced expression of the fibrinolytic stimulator, tissue-type plasminogen activator (tPA), and was partly due to an increase in the main inhibitor, plasminogen activator inhibitor type 1 (PAI-1)[8,9]. Moreover, patients who developed severe adhesions after surgery had significantly raised levels of peritoneal PAI-1 compared with normal subjects[10].

Thus, the production and release of plasminogen activator inhibitors is believed to be a major factor in the loss of peritoneal fibrinolytic activity and inhibition of PAI-1 might therefore be beneficial. Until now, PAI-1 inhibition has not been investigated for its potential to reduce adhesion formation.

PRAP-1 is a fragment for antigen binding (Fab) of a PAI-1-inhibiting polyclonal antibody. Inhibition of PAI-1 by PRAP-1 has been shown to significantly enhance fibrinolysis[11]. The aim of this study was to test the hypothesis that inhibition of PAI-1 activity could reduce adhesion formation after surgery.

Materials and methods

Animals

Fifty-three female NMRI mice weighing 25–30 g were used. The experiments were approved by the local ethics committee and animals were kept according to standard guidelines. The animals were given a standard pellet diet and water *ad libitum*, before and after surgery.

PRAP-1

PRAP-1 (Fab fragments of polyclonal rabbit PAI-1 antibody) was kindly provided by AstraZeneca Research and Development (Umeå, Sweden). PRAP-1 had been prepared from rabbits immunized with recombinant human PAI-1 produced in mammalian cells. The immunoglobulin (Ig) G fraction obtained was cleaved with papain. Fc fragments (the effector region) and uncleaved antibodies were removed by affinity chromatography on Protein A–Sepharose (Pharmacia Biotech, Uppsala, Sweden), and Fab fragments were concentrated by ultrafiltration. A Fab fragment of a control antibody IgG has been shown previously not to affect PAI-1 activity[12].

Surgical technique

Mice were anaesthetized by inhalation of methoxyflurane (Metofane; Pitman Moore, Mundelein, Illinois, USA). The peritoneal cavity was accessed via a midline incision and a 2 × 15 mm area of the parietal peritoneum on each lateral abdominal wall was excised. The peritoneal lesions were closed with four 4/0 sutures and the midline incision in one layer with a running 3/0 Dexon suture (Davies and Geck, St Louis, Missouri, USA). The procedure has been described further elsewhere[13].

Experimental design

At the end of the operation, 0·75 ml of a saline solution containing PRAP-1 15 mg/kg ($n = 18$) or 30 mg/kg ($n = 17$) or, as a control, saline solution only ($n = 18$) was administered intraperitoneally just before closure of the midline incision.

Evaluation

The presence of adhesions was assessed 7 days after operation. This has been demonstrated previously to be a suitable time point for this model[13–15]. The animals were given an overdose of isoflurane (Forene; Abbott Laboratories, Queensborough, England), and the abdomen was opened widely so that the peritoneal lesions created previously, and adherent structures, were exposed. The adhesion attachments were measured by an observer blinded to the previous treatment. The proportion of the peritoneal lesion they covered was calculated and expressed as a percentage of the original lesion. In addition, the number of attachments to different structures was noted. The method of measurement has been described in detail elsewhere[13].

Statistical analysis

After determining an overall difference in adhesion formation between groups with the Kruskal–Wallis test, the non-parametric Mann–Whitney *U* test was used to compare the groups. $P < 0{\cdot}05$ was considered statistically significant.

Results

All animals survived and all developed adhesions. There were no signs of bleeding in the postoperative period or at reoperation. There was a significant overall difference in adhesion formation between the groups ($P = 0{\cdot}002$). The area of induced peritoneal injury was covered by adhesions to a median level of 64 (95 per cent confidence interval (c.i.) 36–94, i.q.r. 23·3) per cent in control animals (*Fig. 1*). The group that received low-dose PRAP-1 had a significantly smaller area of adhesions (50 (95 per cent c.i. 18–76, i.q.r. 20) per cent; $P = 0{\cdot}003$). Similarly, the group that had high-dose PRAP-1 also had a reduction in adhesions (50 (95 per cent c.i. 12–76, i.q.r. 28·1) per cent; $P = 0{\cdot}002$).

Because adhesions affecting certain organs (e.g. small bowel, female genital tract) might be more important clinically than others, the distribution of adhesions in the peritoneal cavity was studied. There was no statistically significant difference in the rate of adhesion formation ($P = 0{\cdot}84$ to omentum, $P = 0{\cdot}094$ to bowel, $P = 0{\cdot}97$ to other sites), even though the PRAP-1-treated groups tended to have fewer attachments to bowel (*Fig. 2*).

Discussion

These results show that topical inhibition of PAI-1 can reduce postoperative adhesion formation. PRAP-1 has been shown to enhance fibrinolysis significantly both *in vitro* and

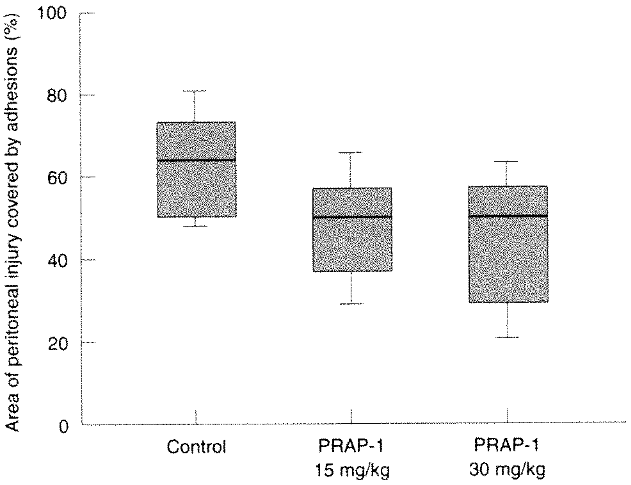

Fig. 1 Adhesion formation after surgery in mice treated with two different doses of PRAP-1 or saline control. Values are median (interquartile range) area of peritoneal injury covered by adhesions

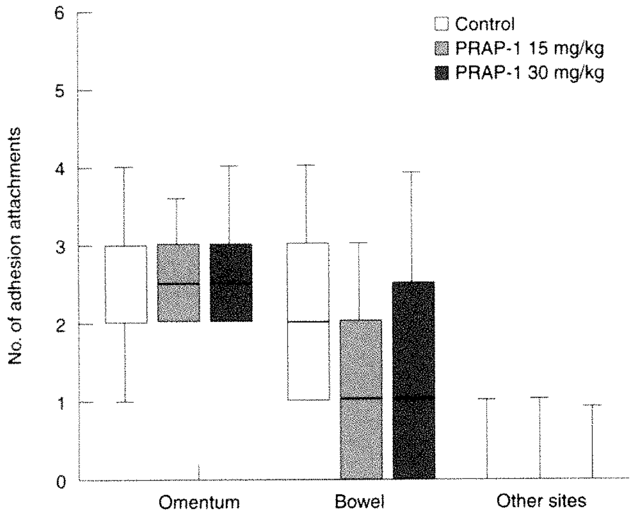

Fig. 2 Number of adhesion attachments between the peritoneal lesion and other structures. Values are median (interquartile range). PRAP-1 tended to reduce the number of attachments to bowel, but the results did not reach statistical significance ($P = 0{\cdot}84$ for omentum, $P = 0{\cdot}094$ for bowel, $P = 0{\cdot}97$ for other sites). Kruskal–Wallis test

in vivo[11]. In rats, PRAP-1 decreased the occurrence of fibrin clots in the lungs, and reduced the thrombus size and restored blood flow in a model of arterial thrombosis[12,16]. It therefore seems reasonable to assume that the action of PRAP-1 in the peritoneal cavity was to enhance fibrinolysis.

In the present study, the low dose of PRAP-1 given intraperitoneally at the conclusion of the operation reduced the formation of adhesions, but there was no significant increase in effect when the dose was doubled. A possible explanation for this is a rapid clearance from the abdominal cavity; PRAP-1 has been demonstrated to stimulate fibrin degradation in a dose-dependent manner[12]. Compounds instilled in the abdominal cavity are known to have a short duration of action if not administered in a slow-release form[17]. The concentrations used could have saturated the abdominal cavity, so that excess PRAP-1 was absorbed by the surrounding tissues. Delivering the antibody in a slow-release form could therefore be a way of increasing its efficacy.

There are significant interspecies differences in the molecular sequence of PAI-1, which might also have affected the results. In this case a rabbit antibody against human PAI-1 was used in a mouse model. In previous studies PRAP-1 was approximately 25 times more potent in inhibiting human PAI-1 than rodent PAI-1[12]. Interspecies differences might explain the limited effect of PAI-1 in the mouse model. Although PRAP-1 significantly reduced adhesion formation, it did not alter the distribution of adhesions.

A substantial part of the fibrinolytic reduction in conditions associated with adhesion formation (e.g. surgery, inflammation, severe adhesion formation) has been observed to be caused by peritoneal overexpression of PAI-1[9,10]. Theoretically, inhibition of PAI-1 is a more controllable and biological approach to increasing local fibrinolytic capacity. Because availability of tPA would then be a rate-limiting factor, local inhibition of PAI-1 might lessen the risk of bleeding complications. However, it cannot be ruled out that systemic absorption of PRAP-1 could lead to bleeding.

The present observations lend further support to the hypothesis of a pivotal role of fibrinolysis in the early formation of adhesions, and open up new possibilities for reducing adhesions by inhibiting PAI-1.

Acknowledgements

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Original article

Review of current practice to establish success after vasectomy

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Background: This study aimed to examine the criteria used by surgeons in a district general hospital to confirm success following vasectomy, to establish the proportion of men undergoing vasectomy in whom the procedure was unsuccessful according to those criteria, and to evaluate their subsequent management.
Methods: All 15 surgeons performing vasectomy indicated that they required two consecutive azoospermic postvasectomy semen specimens before they advised couples that the vasectomy was successful. Results of postvasectomy semen analysis (PVSA) for all 240 primary vasectomies performed over a 12-month interval were analysed. Minimum follow-up was 30 (range 30–42; median 37) months.
Results: At follow-up 72 men (30 per cent) had not returned postvasectomy samples that fulfilled the criteria, including 18 who were azoospermic on the first PVSA 3 months after vasectomy but who failed to produce a second specimen. In 24 men (10 per cent) who failed to comply with the PVSA protocol, there was no documentation of any further action being taken. No pregnancies were reported in the partners of the study group during this interval and only one patient underwent repeat vasectomy.
Conclusion: The results suggest that the strict requirement of two consecutive azoospermic postvasectomy semen specimens may be unjustified, leads to a high level of non-compliance and causes unnecessary delay in confirming success of the procedure.

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Introduction

Vasectomy is a safe, effective and popular method of contraception. Adequate counselling is essential and couples should be advised that analysis of postvasectomy semen specimens is required to confirm success before the use of alternative contraception is abandoned. The protocol used for postvasectomy semen analysis (PVSA) should confirm clearance and identify, as quickly as possible, men who fail to become sterile through either technical failure or early recanalization. The timing of PVSA and the number of specimens required to confirm success remain controversial because of delayed clearance of residual sperm from the ampulla of the vas deferens in some individuals. The current practice in a district general hospital was examined in order to establish the criteria used by individual surgeons to declare success and to determine the proportion of men in whom vasectomy was not successful according to those criteria, and their subsequent management.

Patients and methods

A confidential questionnaire was sent to all surgeons ($n = 15$) performing vasectomy in a district general hospital. They were asked to describe their technique of vasectomy and the criteria they looked for on PVSA to declare 'success'. These were ascertained by their responses to case scenarios and by direct questioning. In the case scenarios, the results of PVSA at various intervals following vasectomy were outlined and respondents were asked to indicate what action they would take. The options offered were to counsel for repeat vasectomy, to request a repeat specimen, or to advise that the vasectomy had been successful. PVSA results were subsequently examined for consecutive vasectomies performed in the hospital within a 12-month interval between April 1995 and April 1996. This allowed a minimum follow-up of 30 months.

Case records were reviewed for all men whose vasectomy was not successful according to locally applied criteria for PVSA. All PVSA was carried out by one microbiologist who investigated 20 fields of non-centrifuged semen samples under the microscope with a × 200 magnification. Results were graded semiquantitatively as showing azoospermia (no sperm), very scanty sperm (fewer than five per high-power field (HPF)), scanty (five to ten per HPF), moderate (ten to 50 per HPF) or numerous (more than 50 per HPF) sperm. The constraints of the laboratory service meant that no assessment of motility was made unless clearly motile sperm were present.

During the study interval 240 primary vasectomies were performed. The median follow-up was 37 (range 30–42) months. All subjects were asked to provide two initial specimens for analysis at 3 and 4 months after the procedure, with subsequent follow-up of men who did not have a clear specimen being left to the individual surgeon's discretion.

Results

Questionnaires

All surgeons responded to the questionnaire. The group comprised three urologists (two consultants, one specialist registrar) and 12 general surgeons (six consultants, six trainees). All 15 reported a requirement for two consecutive azoospermic specimens to confirm success. In addition all respondents stated that if either of the initial two specimens showed any spermatozoa they would ask patients to undergo further PVSA until azoospermia was documented on two consecutive samples.

Success

At least one specimen for PVSA was provided by 206 men (86 per cent) and 184 (77 per cent) returned at least two specimens. Some 168 (70 per cent) eventually met the established criteria for success (two consecutive azoospermic postvasectomy semen samples) after a median of 2 (range 2–14) samples. Of the 72 men (30 per cent) not fulfilling the established criteria for success, 18 were azoospermic on the first PVSA 3 months after surgery, but failed to produce a second specimen. Establishing success by these criteria took a median of 143 (range 92–532) days. Of the 168 men who eventually returned two consecutive azoospermic specimens, 36 (21 per cent) had returned an initial (first or second) azoospermic sample but were asked to return a further specimen because the other specimen showed scanty or very scanty sperm. In eight of the 36, clearance was delayed for up to 12 specimens (18 months).

Of the 184 men who returned two or more samples, 16 (9 per cent) did not meet the applied criteria for 'success'. In two (1 per cent), the vasectomy was deemed to have failed and the men were offered a repeat procedure. Only one of these two elected to undergo repeat vasectomy; the second had separated from his partner by the time he responded. Four of the 16 men were eventually advised that they could consider themselves infertile on the basis of intermittent azoospermic specimens, and the remaining ten did not respond to letters requesting they return further specimens. Thus, the overall number of men who received confirmation of success following a single procedure was 172 (72 per cent).

Compliance

Thirty-four men (14 per cent) failed to return any samples for PVSA and 22 (9 per cent) returned only one initial sample. A further ten (4 per cent) did not respond to requests for repeat specimens, having provided at least three samples. This gave a non-compliance rate of 28 per cent, based on failure to respond to a request for two consecutive azoospermic specimens.

Azoospermia and recurrence of spermatozoa

Of the 206 men who returned specimens for PVSA, a total of 196 men (95 per cent) were azoospermic on at least one specimen. In 154 (74 per cent) this was on the very first PVSA. The cumulative frequency of men achieving success plotted against the number of specimens provided is shown in *Fig. 1*. Of the 184 men who provided at least two specimens for PVSA, 15 (8 per cent) had reappearance of spermatozoa at some stage during follow-up, having provided at least one azoospermic specimen. Eight of these men went on to produce two consecutive azoospermic specimens at varying intervals following vasectomy. All of these eight men had produced a single azoospermic specimen by the time of their third sample but were requested to provide a further one to nine samples before the operation was deemed successful. Of the remainder

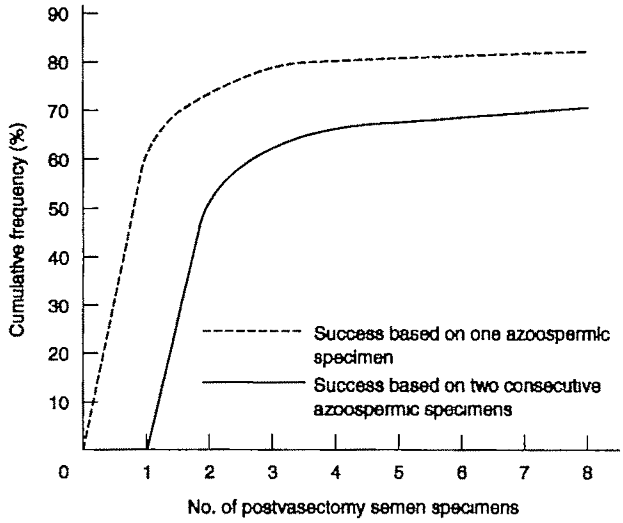

Fig. 1 Cumulative frequency of men achieving success following vasectomy

($n = 7$), four were advised they could discontinue other methods of contraception and three defaulted on follow-up, despite reminders.

Case record review

In all, 72 men failed to provide two consecutive azoospermic postvasectomy specimens as required by the locally applied criteria for success. In two, the vasectomy was deemed to have failed and a repeat procedure was offered, as stated previously. Four others were sent a letter recommending discontinuation of alternative contraceptive techniques, based on the last PVSA showing azoospermia. Forty-two (58 per cent) did not respond to reminders requesting further specimens. In several of these cases, a copy of the letter was sent to the general practitioner. In 24 (33 per cent) there was no record of any action being taken, in the form of either a reminder to the patient or a letter to the general practitioner. Of these, two had a positive PVSA (both very scanty) and eight had azoospermia recorded for the last specimen. Fourteen had never provided a specimen for PVSA.

Discussion

The traditional protocol for PVSA requires two consecutive azoospermic specimens at 12 and 16 weeks postvasectomy[1,2]. There is no evidence to suggest that insistence on two specimens, rather than a single one, reduces the risk of subsequent pregnancy. Indeed sperm can be demonstrated in the semen of up to 10 per cent of men presenting for vasectomy reversal following documented azoospermia[3]. The only aim of documenting azoospermia in the early postoperative period is to confirm division of both (all) vasa. This does not guarantee success in the long term; couples must be advised of the small risk of late recanalization and subsequent pregnancy. The impracticality of the current guidelines was revealed by the fact that they were being ignored, both explicitly by patients (56 failed to return two specimens) and occasionally implicitly by surgeons. Four men were told they could consider the vasectomy a success despite not having produced two consecutive azoospermic specimens. A further eight men who had one azoospermic specimen on record were not requested to bring repeat specimens.

These guidelines also impose unnecessary delay in stipulating success in some men. Despite having initially proven azoospermia, eight men repeated specimens up to 12 times before the vasectomy was deemed successful, without any further intervention. The aim of vasectomy is to confer peace of mind to a couple who have decided their family is complete; to delay this by 12–18 months after the vasectomy seems unfair.

The protocol of two consecutive azoospermic specimens was found to present barriers to compliance. The initial non-compliance rate (i.e. those not returning any samples at all) of 14 per cent in the present study was similar to rates of 24–36 per cent reported previously in the USA[4] and the UK[5]. However, non-compliance increased to 28 per cent when based on failure to respond to a request to produce two consecutive azoospermic specimens. Responsibility for non-compliance is a contentious issue. Preoperative and perioperative counselling alone may be insufficient to protect a surgeon from accusations of negligence if pregnancy occurs in the partners of men who do not have documented success[4]. Advice about the need for PVSA should be documented and those who do not comply should be identified and followed up.

The issue of reappearance of small numbers of non-motile sperm is contentious. A survey of British urologists showed marked variation in the management of patients whose specimens show persistence or reappearance of non-motile sperm[2]. The frequency of reappearance of sperm following a single azoospermic specimen has been documented as around 9 per cent[6] and as about 5 per cent at 12 months after two azoospermic specimens[1]. The value of 8 per cent reported here is consistent with the literature[3]. There have been reported cases of paternity confirmed on DNA testing, despite total azoospermia documented both before and after the date of conception[7,8]. Edwards[9] argues that the presence of non-motile sperm is irrelevant, suggesting earlier testing with analysis of motility. This approach may not be practical in the UK, given the limitations in determining intervals between production and analysis of the specimen. The Oxford group[10] has shown that a special clearance category can be advised provided that two consecutive specimens have counts lower than 10 000 per ml and at least 7 months have elapsed since vasectomy. In their study of 151 men given special clearance, 50 responded to follow-up at 3 years and all but one were azoospermic at that point. There were no reported pregnancies in that group.

The strict requirement for two consecutive azoospermic samples may be unjustified and results in repetition of PVSA. A single PVSA at 4 months postvasectomy should allow earlier confirmation of technical success, improve compliance and facilitate earlier detection of non-compliers without any decrease in the detection of operative failure.

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Original article

Incidence of acquired immune deficiency syndrome (AIDS)-related disorders at laparotomy in patients with AIDS

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Background: Patients with acquired immune deficiency syndrome (AIDS) present for surgical management of abdominal conditions that are complications of advanced human immunodeficiency virus (HIV) infection or that are caused by other disease not related to AIDS. This study compared the clinical details and postoperative outcomes of patients with AIDS-related diseases found at laparotomy with those of patients with non-AIDS-related disorders.

Methods: The medical records of 30 consecutive patients with AIDS who underwent laparotomy were examined retrospectively.

Results: Fourteen patients had AIDS-related pathologies found at laparotomy, 13 had disease processes that were not AIDS related and three had no abnormal findings at laparotomy. Patients with AIDS-related conditions at laparotomy had lower mean body-weight, serum albumin concentration and CD4 lymphocyte count, and required a longer hospital admission than those who had non-AIDS-related disease. The duration of HIV infection and the number of complications and deaths were similar in the two groups. Complications occurred in 21 patients and there were five deaths (30-day mortality rate 17 per cent).

Conclusion: AIDS-related pathologies are commonly found at laparotomy in patients with HIV/AIDS. An AIDS-related diagnosis does not confer a greater risk of complication or postoperative death than other conditions.

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Introduction

Human immunodeficiency virus (HIV) infection is a major health issue, with high rates of infection and subsequent development of the acquired immune deficiency syndrome (AIDS) in many countries[1]. Patients with HIV or AIDS may present to the surgeon for evaluation of abdominal pain or suspected malignancy. The underlying cause may be secondary to the immunodeficient state or may be unrelated to the HIV infection. Opportunistic infections and malignancies characterize the abdominal complications of AIDS, often mimicking more conventional diagnoses. In many cases the management of such complications is non-operative. Previous studies that have examined the role of laparotomy in patients with AIDS have not examined the effects of such AIDS-related pathologies on outcome after surgery, and have not examined risk factors associated with such conditions that may assist in earlier preoperative identification.

The records of patients with AIDS who underwent laparotomy at this hospital were examined retrospectively. The preoperative clinical details and postoperative outcomes of patients with AIDS-related pathologies found at surgery were compared with those of patients whose disease processes were unrelated to HIV infection.

Patients and methods

The medical records of all patients who were seropositive for HIV infection, met the Centers for Disease Control (CDC) criteria for AIDS[2] and who underwent laparotomy between January 1987 and December 1998 were reviewed retrospectively. Only patients undergoing midline laparotomy were included in the study; patients who underwent appendicectomy or cholecystectomy via a different surgical approach were excluded.

Age, sex, body-weight, past medical history, risk factors for HIV exposure, current medical treatment, preoperative diagnosis, operative findings, results of histopathology and details of postoperative complications or deaths were recorded. All patients had full blood count and serum biochemistry tests, including serum albumin concentration, performed on admission. All patients had a CD4 T-lymphocyte count performed within 6 months of admission.

Patients with an AIDS-related disease process diagnosed at laparotomy were compared with those who had findings unrelated to the HIV infection. A diagnosis was considered AIDS related if it met the CDC definition of an AIDS-defining condition[2].

Results were analysed by standard statistical methods. Numerical results are presented as mean (95 per cent confidence interval) unless otherwise stated.

Results

Laparotomy was carried out on 30 patients with AIDS during the 12-year period January 1987 to December 1998. Patient age was 38 (35–41) years. There were 29 men and one woman.

Details of human immunodeficiency virus infection

Risk factors for HIV exposure were homosexual intercourse in 20 patients and intravenous drug use in two; in the remaining eight patients no risk factors were documented in the admission records. The mean time from diagnosis of HIV infection to laparotomy was 7 (range 0–14) years. Eighteen patients had a previous AIDS-defining condition. A further four patients had their first AIDS-defining condition identified at laparotomy and were diagnosed with AIDS during the surgical admission. The remaining eight patients had no history of AIDS-defining conditions, but had CD4 T-lymphocyte counts of less than 200 cells/μl and so met the CDC criteria for AIDS[2]. The mean CD4 T-lymphocyte count was 157 (86–228) cells/μl. Four patients gave a history of infection with hepatitis B virus and two with hepatitis C.

Laparotomy

Tables 1 and *2* show the preoperative indications for laparotomy and the final diagnoses after review of the histopathology results and operative findings. Fourteen patients had AIDS-related conditions found at laparotomy while 13 had conditions that were not AIDS related. The clinical details of these two groups are compared in *Table 3*. The remaining three patients had no abnormal findings at laparotomy; two presented acutely with generalized abdominal pain and one underwent elective splenectomy for splenic vein thrombosis but no such abnormality was demonstrated at operation. AIDS-related pathologies accounted for seven emergency and seven elective laparotomies. Eleven patients were found to have intra-abdominal lymphomas at laparotomy, all of which were B-cell non-Hodgkin's type. Four of these lymphomas were found in the small bowel, three in the large bowel, two in the retroperitoneum, and one primarily involved the liver and one the spleen. Five of the lymphomas presented acutely and were diagnosed at emergency laparotomy, two as intestinal perforations, one with profuse colonic bleeding, one as a

Table 1 Preoperative indications in patients with acquired immune deficiency syndrome who underwent laparotomy

Preoperative indication	No. of patients
Acute admissions	
Perforated viscus	10
Small bowel obstruction	3
Intra-abdominal abscess	2
Hepatic/splenic haemorrhage	2
Colonic haemorrhage	1
Elective admissions	
Splenectomy*	5
Large bowel malignancy	3
Open biopsy of liver/spleen	2
Reversal of colostomy	1
Invasive anal SCC†	1

*Splenectomy performed electively for thrombocytopenia, lymphoma, splenomegaly, splenic vein thrombosis and splenic infarcts. †Abdominoperineal resection. SCC, squamous cell carcinoma

Table 2 Postoperative findings in patients with acquired immune deficiency syndrome who underwent laparotomy

Postoperative diagnosis	No. of patients
AIDS-related conditions	
Lymphoma	11
Kaposi's sarcoma	2
CMV acalculous cholecystitis	1
Non-AIDS-related conditions	
Perforated appendicitis	3
Benign splenomegaly	2
Perforated colonic diverticulitis	1
Colonic ischaemia	1
Traumatic splenic rupture	1
Abdominal adhesions	1
Traumatic rectal perforation	1
Splenic infarcts	1
Haemorrhage after needle biopsy of liver	1
Invasive anal SCC	1
No abnormal findings at laparotomy	3

AIDS, acquired immune deficiency syndrome; SCC, squamous cell carcinoma; CMV, cytomegalovirus

Table 3 Clinical details and postoperative outcome of patients with acquired immune deficiency syndrome (AIDS)-related and non-AIDS-related conditions found at laparotomy

	AIDS related (n = 14)	Non-AIDS related (n = 13)
Age (years)*	38 (33–43)	38 (32–44)
Time from HIV diagnosis (years)*	6 (4–8)	8 (5–10)
Body-weight (kg)*	58 (52–64)	71 (65–78)
Previous AIDS-defining illness	7	9
Type of admission		
Emergency	7	9
Elective	7	4
CD4 lymphocyte count (cells/µl)*	122 (23–221)	205 (68–342)
Serum albumin (g/l)*	33 (30–37)	37 (34–40)
No. with complications	8	10
No. of deaths	3	2
Duration of admission (days)*	21 (16–25)	14 (9–19)

*Values are mean (95 per cent confidence interval). Three patients had no abnormal findings at laparotomy. HIV, human immunodeficiency virus

perinephric abscess and one resembling an appendiceal abscess. Kaposi's sarcoma was identified in two patients; one presented acutely with small bowel obstruction from intraperitoneal spread of the malignancy and one with a large splenic tumour deposit at splenectomy. One patient presented with acalculous cholecystitis secondary to cytomegalovirus (CMV) infection and cholecystectomy was performed.

Morbidity and mortality

The duration of admission was 17 (14–20) days, and was longer for patients who had AIDS-related pathologies found at laparotomy than for those who had other causes (*Table 3*). Thirty-two complications occurred in 21 patients. Infectious complications were encountered most frequently. Ten patients developed a chest infection that required parenteral antibiotics. Four patients developed systemic sepsis (one of whom became coagulopathic), four patients developed wound infections and one patient developed an intra-abdominal abscess. Incisional hernias were found in three patients on follow-up examination, and one patient had an abdominal wound breakdown. Epileptic seizures occurred in three patients during hospital admission, only one of whom had a history of epilepsy (secondary to cerebral toxoplasmosis). Other complications observed were prolonged postoperative ileus in two patients, sacral pressure ulceration in one patient and acute pulmonary oedema in one patient. Five deaths occurred during admission for laparotomy (30-day hospital mortality rate 17 per cent); four were secondary to overwhelming systemic sepsis and one was due to coagulopathy. The incidence of complications and death after laparotomy for AIDS-related causes was similar to that after laparotomy at which other disease processes were found (*Table 3*). Complications developed in all three patients who had no abnormal findings at laparotomy (chest infection, wound infection and incisional hernia) but there were no postoperative deaths.

Discussion

HIV infection is a major cause of death. The number of cases of HIV infection and AIDS is increasing in many nations[1]. In Australia to the end of 1998, 17 600 patients were diagnosed with HIV and 8071 with AIDS[3]. Most were males who contracted HIV infection from homosexual intercourse[3].

HIV infection causes a gradual decline in helper T-lymphocyte numbers, predisposing to infection by opportunistic organisms and the appearance of malignancies that characterize the clinical syndrome of AIDS[4]. Patients with HIV infection may present to the gastrointestinal surgeon for evaluation or management of clinical problems, including abdominal pain and abdominal emergencies, or with suspected malignancy of the gastrointestinal tract. The underlying cause may be specific to HIV/AIDS, or may be secondary to a condition not related to AIDS. In the present study, AIDS-related pathologies accounted for almost half the laparotomies performed in patients with AIDS. The most common condition was intra-abdominal B-cell non-Hodgkin's lymphoma that presented acutely with perforation, as an abdominal mass or with organomegaly. Surgeons investigating abdominal symptoms in patients with AIDS should always consider the diagnosis of intra-abdominal lymphoma, as presentation is variable and may mimic other more conventional conditions. Kaposi's sarcoma was less frequent, occurring in only two patients, and only one patient presented with intra-abdominal complications of CMV infection. Other reports show a similar percentage of AIDS-related pathologies found at laparotomy[5–10]. However, there were more intra-abdominal CMV and mycobacterial (*Mycobacterium avium intracellulare* and *M. tuberculosis*) infections in many of these series[6–10].

Body-weight, serum albumin concentration and CD4 lymphocyte count were lower in patients with an AIDS-related pathology found at laparotomy than in those in whom non-AIDS-related causes were present. The debilitating effects of AIDS-related malignancy or opportunistic infection may explain this, or these findings may be a marker for the more advanced stage of HIV/AIDS in these patients. In any case, clinicians should suspect AIDS-related diagnoses such as lymphoma when there is significant weight loss, low serum albumin concentration or CD4 lymphocyte count. Patient age, time since diagnosis of HIV infection and past history of AIDS-defining conditions were similar in the two groups.

There were more urgent than elective laparotomies for patients with AIDS, as might be expected given that the patients were predominantly male and relatively young. An AIDS-related condition was found to be the underlying cause in seven of 18 patients who underwent emergency laparotomy, including a number with intestinal lymphoma presenting with perforation. In three patients there were no abnormal findings at laparotomy. Given the high morbidity and mortality rates associated with major abdominal surgery in patients with AIDS, every effort should be made to avoid unnecessary laparotomy by appropriate investigation and consideration of medical diagnoses. Computed tomography, ultrasonography of the abdomen and laparoscopy have been suggested as useful investigations when the cause of abdominal pain in patients with HIV infection is unclear[11].

Most of the complications seen after laparotomy in patients with AIDS were due to sepsis, particularly pulmonary and wound infections. There was also a significant number of patients with incisional hernia or wound breakdown. These high complication rates are similar to those in other reports of patients with AIDS undergoing major abdominal surgery[5–9,12–15], and reflect the presence of comorbidity from opportunistic infections such as *Pneumocystis carinii* pneumonia, and nutritional and metabolic deficits. Low preoperative CD4 T-lymphocyte counts and emergency laparotomy have been associated with an increased likelihood of complications[6,9]. The morbidity and mortality rates observed after laparotomy for AIDS-related and non-AIDS-related disorders were similar, although patients with AIDS-related diseases required a longer hospital stay, mostly because of ongoing medical management issues.

Surgeons who have to manage abdominal conditions in patients with AIDS, both acute and elective, should be aware of the relative frequency of AIDS-related malignancy and infection found at surgery in these patients. Given the relatively high rates of morbidity and mortality associated with laparotomy in these patients, surgeons should consider conservative and medical management wherever possible.

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Effect of CD4+ and CD8+ cell depletion on wound healing

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Background: Depression of the immune system can result in poor or delayed wound healing.
Methods: Thymectomized rats were depleted of CD4+ and CD8+ lymphocytes by intraperitoneal injection of Medical Research Council Oxford (MRC OX)38 antibodies and MRC OX8. Significant depletion was demonstrated throughout the wound healing process by immunofluorescence studies of peripheral blood. Following depletion the rats underwent laparotomy incisions which were allowed to heal for 10 weeks. Differences in healing were demonstrated by analysing the wounds biomechanically by tensiometry to obtain values of ultimate strength, resilience, toughness, maximum extension and elastic constant.
Results: Wounds of animals depleted of CD4+ lymphocytes showed a significant decrease in ultimate strength, resilience and toughness. Wounds of animals depleted of CD8+ lymphocytes showed a significant increase in ultimate strength, resilience and toughness.
Conclusion: Wounds healed in the absence of T lymphocytes. However, the subsets have an opposing regulatory role, with CD4+ lymphocytes upregulating and CD8+ lymphocytes downregulating wound healing.

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Introduction

The participation of lymphocyte subpopulations in wound healing is a dynamic and distinctive process which has been shown in wounds of rodents[1] and humans[2]. T lymphocytes produce a number of factors that affect both macrophages and fibroblasts, both of which have major roles in wound healing. Direct evidence for lymphocyte involvement in the control of wound healing is provided by both clinical conditions and laboratory studies.

A number of clinical conditions associated with a depression of the underlying immune function, such as diabetes mellitus[3], acquired immune deficiency syndrome[4], jaundice[5], advanced age[6] and radiotherapy[7], are subject to wound healing problems. In addition patients on immunosuppressive treatment are at risk of impairment of wound healing[8–10].

Previous studies have investigated the effect of depletion of T lymphocytes on wound healing in mice by means of specific cytotoxic monoclonal antibodies[11–13]. It has been demonstrated that, whereas overall depletion of T lymphocytes results in impaired wound healing, specific depletion of CD4+ and CD8+ T lymphocyte subsets, either alone or in combination, produces varying and conflicting results with either weaker or stronger wounds. These studies have shown that a reduction in T lymphocyte numbers alters the properties of a wound after healing, but the results are confusing in terms of the opposing effects of lymphocyte subsets. In all of these studies only a reduction in T lymphocyte numbers was demonstrated, rather than complete depletion, and the animals had to undergo continual treatment with injection of xenogeneic antibodies throughout the course of wound healing.

The present study was undertaken to measure a wider range of parameters in the analysis of wound healing and to determine the effect of significant depletion of CD4+ and CD8+ lymphocytes on this process at a time in which wounds should have healed completely.

Materials and methods

Animals, maintenance and treatment

All animal experiments were carried out in accordance with the Animal (Scientific Procedures) Act 1986. Six-week-old PVG rats were obtained from Harlan® UK Ltd (90–120 g) and allowed to acclimatize for 2 weeks following importation into the designated animal establishment. These animals were chosen for their relatively larger size, lack of excessive subcutaneous tissue and ease at producing adequate wound material for biomechanical assessment compared with mice.

Rats were thymectomized and randomized into four groups (control, CD4$^+$ depletion, CD8$^+$ depletion, and CD4$^+$ and CD8$^+$ depletion). A sample size of 32 rats in each group was used, as determined statistically from data obtained in a small pilot study.

Depletion of either CD4$^+$ and CD8$^+$ cells was effected as follows. For CD4$^+$ cell depletion, PVG rats weighing less than 200 g were given two subsequent intraperitoneal injections containing 1 mg MRC OX38 in ascites[14] with a 7-day interval between injections. Similarly, for CD8$^+$ cell depletion rats were administered with 1 mg MRC OX8 by two subsequent intraperitoneal injections[14]. One week after the treatment, a peripheral blood sample was obtained for a prewounding CD4$^+$ and CD8$^+$ lymphocyte count to ensure the efficacy of the depletion in each rat.

Following the treatment regimen and confirmation of T cell depletion, laparotomy was performed under general anaesthesia in all rats. Laparotomy was carried out using bilateral paramedian incisions 5 cm long and 1 cm from the midline in the skin from the costal margin to the lower abdomen, and closed by means of a standard surgical technique[15]. All rats were killed 70 days after laparotomy; before death the animals were weighed at regular intervals and the wounds checked for evidence of dehiscence or infection. Any animal showing signs of distress at any time was killed. A blood sample was obtained via cardiac puncture at post-mortem examination in order to ascertain the long-term effect of the depletion regimen on the CD4$^+$ and CD8$^+$ lymphocyte count. Animals that did not show more than 95 per cent depletion of the specific target T lymphocytes at the time of killing were discounted from the study.

The wounds were harvested by excision of the anterior abdominal wall for the determination of biomechanical properties by tensiometry. Wounds that had developed total wound dehiscence were discounted from the study.

Collection and analysis of blood samples by immunofluorescence

Blood was taken into ethylenediamine tetra-acetic acid (final concentration approximately 5 mmol/l) before thymectomy and laparotomy, and at the time of death. Blood samples (50 µl per tube) were stained with anti-rat CD4, phycoerythrin (clone MRC OX35), and anti-rat CD8-β, fluorescein isosthiocyanate (FITC) (clone 341), or the appropriate isotype control antibodies (clone F10-89-4 or clone F8-11-13) (Serotec, Oxford, UK). The samples were incubated at 4°C for 30 min, and the red cells were lysed with 2 ml lysing solution (Ortho-mune^{TM} lysing reagent). The samples were analysed within 2 h by flow cytometry using the Ortho cytoron absolute^{TM} laser flow cytometer. (Ortho Diagnostic Systems, Raritan, NJ, USA).

Determination of wound biomechanics by tensiometry

The rats were killed and the musculofascial component of the laparotomy wounds was prepared for biomechanical assessment by tensiometry using the Instron 4301 universal materials testing instrument (Instron, High Wycombe, UK). This component was used for the ease of identification of the exact site of the wound, ease of handling for biomechanical assessment, and its clinical relevance relating to the inherent strength the laparotomy wound. The wounds were analysed fresh[16] and within 2 h of excision to minimize the effect of ischaemic autolysis. The central portions of the wounds were divided into multiple segments, each 1 cm in length, for biomechanical testing; uniformity of the cross-sectional area was confirmed with vernier callipers. The specimen was held by pneumatic grips with a serrated surface operated by a portable compressor (grip pressure 75 p.s.i.). The distance between the clamps was the specimen gauge length and was set at 10 mm. Using the stay sutures, each strip was clamped securely in the jaws of the pneumatic clamps so that the wound was midway between the sites of fixation and aligned to avoid shearing forces[17]. The cross-head speed was set at 10 mm/min and the specimen subjected to a constant rate of uniaxial extension to produce complete disruption of the wound. The point of rupture of the specimen was noted carefully to ensure this conformed to the site of the wound.

The complexity of wound biomechanics means that description based on only one parameter may be misleading[18]. Quantitative properties can be calculated from the load *versus* extension curve (*Fig. 1*). The mechanical properties below summarize how a wound responds to load and deformation[19].

Ultimate strength. Ultimate strength (N) is the maximum tension a wound is capable of developing before rupture. It is the maximum ordinate on the load *versus* extension curve where the tangent to the curve is horizontal.

Maximum extension. Maximum extension (mm) is the displacement at the point of wound rupture. It is the maximum abscissa on the load *versus* extension curve.

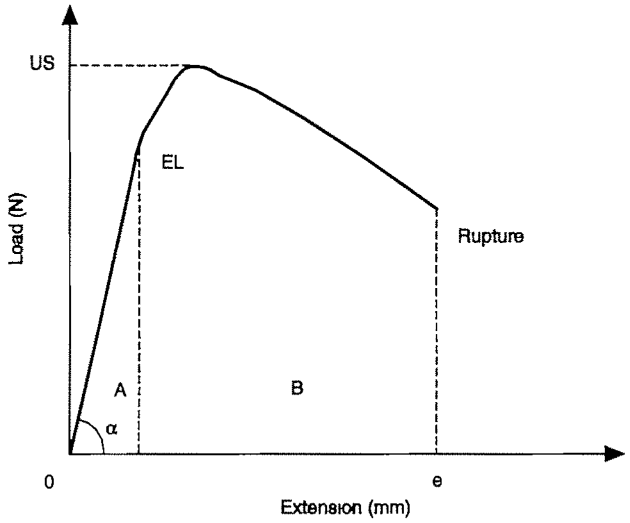

Fig. 1 Representation of the load *versus* extension curve for a wound to demonstrate the derivation of biomechanical parameters. US, ultimate strength (N); e, total extension (mm); area A, resilience (J); area A + B, toughness (J); α, elasticity constant (N/mm); EL, elastic limit (the greatest load the wound is capable of resisting without permanent deformation)

Toughness. Toughness (J) is the property of a material that enables it to endure loads. When a material is subject to a load, some of the energy is transmitted and absorbed by the material. The toughness is the energy absorbed, which is the product of the maximum extension and mean force, up to the point of rupture. It is equal to the total area under the load *versus* extension curve.

Resilience. Resilience (J) is the property of a material that enables it to endure loads without inducing a tension exceeding the elastic limit. It implies that the energy absorbed by the material is stored and released when unloaded. It is equal to the area under the elastic portion of the load *versus* extension curve from the origin to the elastic limit.

Elasticity constant. The elasticity constant (N/mm) is the force applied per unit extension for the material up to the elastic limit. It is a measure of stiffness and resistance to deformation, and is obtained from the slope of the linear portion of the load *versus* extension curve.

Statistical analysis

Differences in initial values of baseline weight and baseline proportion of CD4⁺ and CD8⁺ lymphocytes in the four groups following randomization before the experiment were compared by one-way analysis of variance. $P < 0.05$ was considered statistically significant.

Comparisons of biomechanical parameters of wounds of the T cell-depleted groups and those of the control group were performed with Student's t test, assuming normally distributed data in each group; $P < 0.05$ was considered significant.

Results

Weight of animals

Animals weighed less than 200 g at the start of the experiment. One-way analysis of variance showed no significant difference in baseline weights ($P = 0.12$) between the four groups.

During the course of the experiment, all animals remained healthy, grew and gained weight. For each group, there was a significant increase in weight from baseline, before operation, 5 weeks after operation and weight at death. The weight gain was irrespective of the immunosuppressive treatment. One rat died at the time of laparotomy while under anaesthesia.

Percentage CD4$^+$ and CD8$^+$ counts in peripheral blood

Table 1 shows the mean(s.e.m.) peripheral CD4$^+$ and CD8$^+$ lymphocyte count for each group at baseline, prelaparotomy and at the time of death. One-way analysis of variance demonstrated no significant difference in baseline CD4$^+$ ($P = 0.37$) or CD8$^+$ ($P = 0.44$) cell count between the four groups. The results show that treatment of thymectomized rats resulted in a significant depletion of the target T lymphocyte subpopulation and that in most cases the lymphocytes did not return.

Treatment of thymectomized rats with MRC OX38 resulted in a significant depletion (more than 95 per cent) of CD4$^+$ lymphocytes. Although initial depletion occurred in all treated animals, reconstitution appeared at the time of death in three animals from the CD4$^+$-depleted group and in two from the CD4$^+$ and CD8$^+$-depleted group. Treatment of thymectomized rats with MRC OX8 resulted in a significant depletion (greater than 95 per cent) of CD8$^+$ lymphocytes. Although initial depletion occurred in all but one treated animal from the CD4$^+$ and CD8$^+$-depleted group, reconstitution appeared at the time of death in two further animals from the CD8$^+$-depleted group. These animals showing the presence of target T lymphocytes before laparotomy or at the time of death were excluded from the statistical analysis.

Biomechanical properties of wounds

Tables 2–4 show the mean(s.e.m.) biomechanical properties of ultimate strength, resilience, toughness, maximum extension and elasticity constant. Values in the control and T cell-depleted groups are compared with Student's *t* test.

Wounds from the CD4$^+$-depleted group (*Table 2*) showed a significantly decreased ultimate strength, resilience, toughness and maximum extension compared with the control group. Wounds from the CD8$^+$-depleted group (*Table 3*) demonstrated a significantly increased ultimate strength, resilience, toughness and elasticity constant compared with the control group. Wounds from the CD4$^+$ and CD8$^+$-depleted group (*Table 4*) showed a significantly decreased resilience, toughness and maximum extension but increased elasticity constant compared with the control group. These results indicate opposing influences of CD4$^+$ and CD8$^+$ cells on wound healing by demonstrating differences in wound biomechanical properties between animals depleted of CD4$^+$ and CD8$^+$ lymphocytes with controls (*Fig. 2*).

Table 1 Lymphocyte counts in each group at baseline, before opeation and death

	Percentage of total lymphocyte count		
	Baseline*	Preoperative	At death
Control (*n*=32)			
CD4$^+$	42·0(0·97)	43·6(0·63)	44·9(0·65)
CD8$^+$	6·6(0·31)	5·1(0·28)	4·5(0·2)
CD4$^+$-depleted group (*n*=32)			
CD4$^+$	44·0(1·07)	0·03(0·01)	0·45(0·24)
CD8$^+$	7·0(0·29)	5·9(0·31)	5·7(0·22)
CD8$^+$-depleted group (*n*=32)			
CD4$^+$	43·9(0·9)	51·1(0·92)	45·4(0·64)
CD8$^+$	7·3(0·28)	0·07(0·02)	0·24(0·08)
CD4$^+$ and CD8$^+$ depleted (*n*=31)			
CD4$^+$	42·6(0·99)	0·04(0·01)	1·5(0·87)
CD8$^+$	7·0(0·28)	0·14(0·03)	0·11(0·03)

Values are mean(s.e.m.). *P = 0·37 for CD4$^+$ count and P = 0·44 for CD8$^+$ count between the four groups (one-way analysis of variance)

Table 2 Effect of CD4$^+$ depletion on wound biomechanical parameters

	Control (*n*=32)	CD4$^+$ depleted (*n*=29)	*P**
Ultimate strength (N)	16·9(0·6)	13·7(0·5)	0·0001
Resilience (J)	0·04(0·002)	0·02(0·002)	< 0·0001
Toughness (J)	0·16(0·005)	0·10(0·006)	< 0·0001
Maximum extension (mm)	20·2(0·5)	16·9(0·6)	0·0001
Elasticity constant (N/mm)	3·5(0·2)	3·8(0·2)	0·23

Values are mean(s.e.m.). *Student's *t* test

Discussion

It can be concluded from the results that depletion of the T cell subsets CD4$^+$ and CD8$^+$ markedly influences the outcome of wound healing in opposing directions.

Depletion of CD4$^+$ lymphocytes results in wounds that have a lower ultimate strength, resilience, toughness and maximum extension compared with controls. Conversely, depletion of CD8$^+$ lymphocytes results in wounds with a higher ultimate strength, resilience, toughness and elasticity constant compared with controls. Predictably, depletion of both CD4$^+$ and CD8$^+$ lymphocytes together results in a wound with properties intermediate between these extremes but differing from controls. It can be deduced from this that the wound healing process is regulated by both CD4$^+$ and CD8$^+$ lymphocytes, which exert opposing actions on the process, but that wound healing still occurs in their absence.

Table 3 Effect of CD8$^+$ depletion on wound biomechanical parameters

	Control (*n*=32)	CD8$^+$ depleted (*n*=30)	*P**
Ultimate strength (N)	16·9(0·6)	20·9(0·6)	< 0·0001
Resilience (J)	0·04(0·002)	0·06(0·003)	0·006
Toughness (J)	0·16(0·005)	0·18(0·006)	0·029
Maximum extension (mm)	20·2(0·5)	19·9(0·7)	0·73
Elasticity constant (N/mm)	3·5(0·2)	4·7(0·3)	0·0005

Values are mean(s.e.m.). *Student's *t* test

Table 4 Effect of CD4$^+$ and CD8$^+$ depletion on wound biomechanical parameters

	Control (*n*=32)	CD4$^+$ and CD8$^+$ depleted (*n*=29)	*P**
Ultimate strength (N)	16·9(0·6)	18·0(0·6)	0·21
Resilience (J)	0·04(0·002)	0·03(0·002)	0·0001
Toughness (J)	0·16(0·005)	0·14(0·006)	0·019
Maximum extension (mm)	20·2(0·5)	18·5(0·6)	0·025
Elasticity constant (N/mm)	3·5(0·2)	5·7(0·3)	< 0·0001

Values are mean(s.e.m.). *Student's *t* test

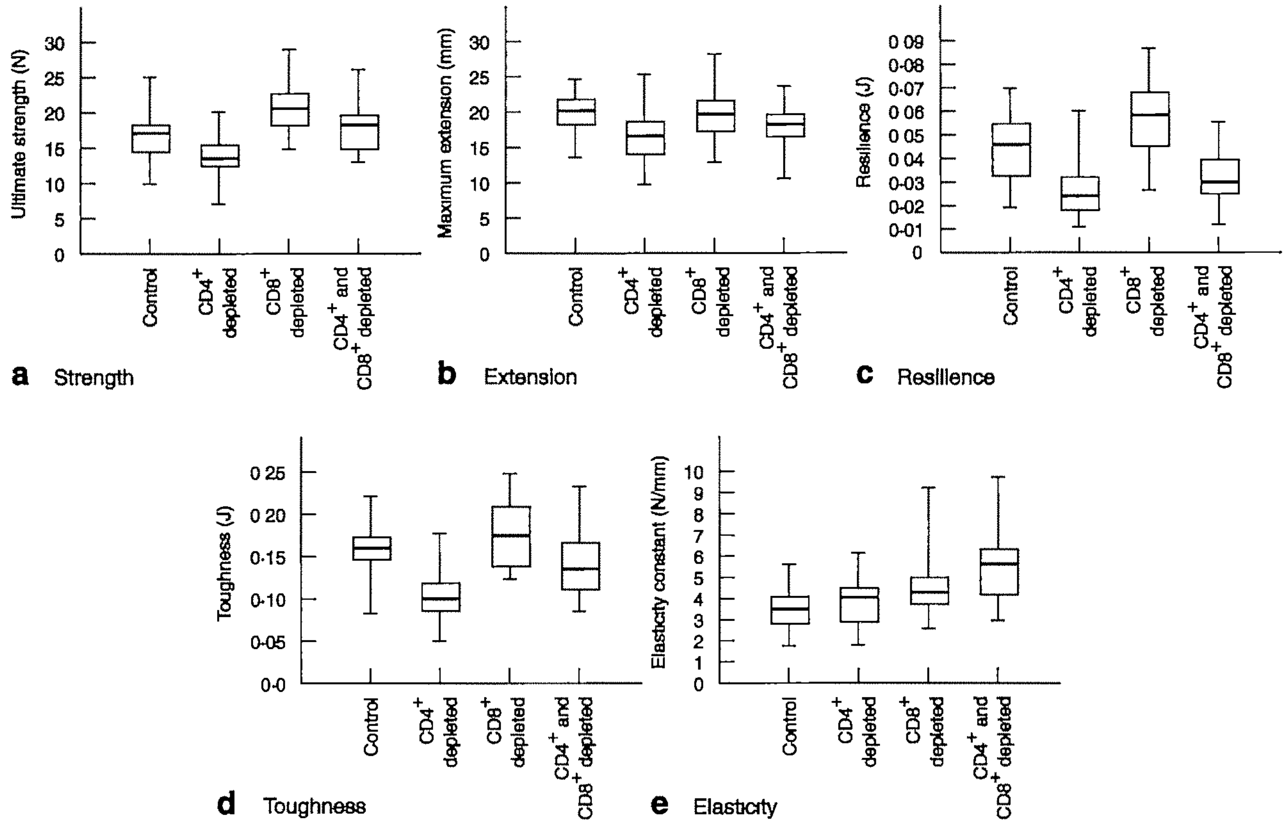

Fig. 2 Effect of T cell depletion on wound biomechanical parameters: **a** ultimate strength; **b** maximum extension; **c** resilience, **d** toughness; and **e** elasticity constant

The lymphocyte subsets CD4$^+$ and CD8$^+$ are subdivided on the basis of the expression of cell surface antigen, but they can also be delineated by analysing the profile of lymphokines released following activation. These lymphokines exert both stimulatory and inhibitory effects on a wide variety of cells, regulating their activity[20]. Principally, in wound healing they affect both fibroblasts (chemotaxis, proliferation and collagen production) and macrophages (chemotaxis, proliferation and monokine production), which are the essential cellular components of wound healing.

In the process of wound healing there is a well defined balance of both stimulatory and inhibitory lymphokines regulating the orderly cellular activity. These factors include lymphocyte-derived chemotactic factor for fibroblasts, fibroblast inhibition factor, fibroblast activation factor, macrophage chemotactic factor, macrophage inhibition factor, macrophage activation factor, interferon γ, transforming growth factor β, interleukin (IL) 1, IL-2 and lymphotoxin[20]. An imbalance of these factors caused by a global depletion of CD4$^+$, CD8$^+$, or CD4$^+$ and CD8$^+$ lymphocytes removes the regulatory influence controlling the activity of fibroblasts and macrophages. This ultimately influences the collagen and proteoglycan content of the extracellular matrix of the wound, resulting in wounds with altered biomechanical properties.

The lymphocytes in wound healing can be thought of as expressing a regulatory role. The process of wound healing continues in the absence of lymphocytes, although their presence is associated with a modulation of the activity of the process to produce either stronger or weaker wounds. CD4$^+$ lymphocytes upregulate the wound healing process as their depletion results in weaker wounds, and CD8$^+$ lymphocytes downregulate the wound healing process as their depletion results in stronger wounds.

Evidence has been presented in the literature for the participation of T lymphocytes and lymphokines in wound healing. Indirectly this comes from histological studies, detection of lymphokines in wounds, investigation of the effects of lymphokines *in vitro* and *in vivo*, investigation of the effects of lymphotropic and lympholytic agents, and the study of wound healing in nude animals, following thymectomy and in the fetus. Direct evidence for lymphocyte involvement in the process of wound healing is provided by studies examining the effects of *in vivo* depletion on wound healing parameters.

These results were expected in part from studies of animal models of mice in which lymphocyte depletion was used to investigate wound healing. Previous studies have looked at the effect of depletion of T lymphocytes in mice using cytotoxic rat antimouse monoclonal antibody 30H12 (anti-Thy1.2)[11]. It was shown that depletion of T lymphocytes in mice impaired wound breaking strength and hydroxyproline deposition. A later study analysed the effect of depleting specific T lymphocyte subsets with rat antimouse monoclonal antibodies[12]. Depletion of $CD4^+$ T cells had no effect on wound healing in terms of wound breaking strength and hydroxyproline deposition, whereas depletion of $CD8^+$ cells enhanced wound healing significantly. A subsequent study depleted T lymphocytes in mice using either pan T cell rat anti-Thy1.2 or a combination of T helper and T suppressor cell depletion with rat antimouse monoclonal antibodies GK1.5 (anti-L3T4) and 2.43 (anti-Lyt2)[13]. It was found that depletion of all T cells (Thy1.2) impaired wound healing whereas depletion of $CD4^+$ and $CD8^+$ cells in combination (L3T4 and Lyt2) enhanced wound healing. It was therefore hypothesized that there must be a subpopulation differentially affected by the treatments which may account for the dissimilar effects. While indicating the importance of T lymphocytes in wound healing, the T lymphocyte depletion in the above studies was not complete. Although T suppressor cells were identified as a downregulator of wound healing, the upregulating component was not established.

Wound healing is impaired in patients with clinical conditions associated with immunodeficiency, such as diabetes and jaundice. These patients, as a result of the underlying condition, often have abnormalities of T lymphocyte function and this may result in an effective downregulation. Patients with human immunodeficiency virus infection and specific $CD4^+$ cell depletion, or those on immunosuppressive regimens leading to $CD4^+$ cell downregulation, may also suffer from impaired healing. The reason for the poor healing in these patients may be the effect of $CD4^+$ cells in wound healing with either low numbers or poorly functioning $CD4^+$ cells. The relevance of these findings is in identifying patients in whom wound healing following operation may be impaired and in determining possible causes, which may be the target of future treatment.

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Original article

Sentinel nodes outside lymph node basins in patients with melanoma

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Background: Lymphoscintigraphy occasionally reveals hot spots outside lymph node basins in patients with melanoma. The aim of this study was to evaluate such abnormally located hot spots.
Methods: Sentinel node biopsy was studied prospectively in 379 patients with clinically localized cutaneous melanoma. One day after lymphoscintigraphy, sentinel node biopsy was performed guided by vital blue dye and a γ ray detection probe.
Results: Persisting hot spots outside the regional node basins were seen in 25 patients (6·6 per cent). Several specific drainage patterns were discerned. In five patients, aberrant sentinel nodes were not explored. The hot spot represented a lymphangioma in two patients. Radioactive lymph nodes were identified in the remaining 18 patients (4·7 per cent). Four patients had metastasis in one of these aberrant lymph nodes.
Conclusion: Sentinel nodes were found outside a lymph node basin in 5 per cent of patients. Particular drainage patterns exist. It is recommended to incorporate such sites in the late scintigraphy images and to pursue aberrant sentinel nodes, as they may be the only sites of metastasis.

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Introduction

A sentinel node is a lymph node to which a primary tumour drains directly. This is the first node to be involved when dissemination occurs. Information on the tumour status of the sentinel node improves the accuracy of staging and provides an opportunity for early regional and systemic adjuvant treatment. Regional lymph node dissection is performed only when metastatic disease is found in the sentinel node. The extent of the clinical benefit of early treatment of patients with melanoma is the subject of several ongoing prospective randomized trials.

Blue dye was used when the sentinel node procedure was first introduced[1]. The γ detection probe and scintigraphy were added later. Lymphoscintigrams drew attention to the fact that sentinel nodes are sometimes situated outside the familiar regional lymphatic basins. The aims of this study were to investigate the incidence, location and tumour status of such aberrant sentinel nodes.

Patients and methods

Between October 1993 and October 1998, 379 patients with melanoma were enrolled into a prospective study on sentinel node biopsy. The study was performed at the Netherlands Cancer Institute/Antoni van Leeuwenhoek Hospital and the University Hospital Groningen. Patients with a clinically localized cutaneous melanoma of at least 1 mm Breslow thickness were included. Pregnant women were excluded. The primary tumours were situated on the leg in 153 patients, on the trunk in 133, on the arm in 58 and in the head and neck region in 35 patients. The same study protocol was used in both hospitals with the approval of the institutional review boards. Informed consent was obtained from all patients.

Lymphoscintigraphy was performed with ^{99m}Tc-labelled human albumen colloid (Nanocoll®; Amersham Cygne, Eindhoven, The Netherlands). A dose of 60 MBq of the radioactive tracer in a mean volume of 0·3 ml was injected intradermally around the tumour or biopsy site. Immediately after injection, dynamic scintigraphy was performed for 20 min using a γ camera with a low-energy,

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The Editors have satisfied themselves that all authors have contributed significantly to this publication high-resolution collimator. Anterior and lateral static images were obtained after 30 min and 2 h. A hot spot was considered to represent a sentinel node if an afferent lymphatic channel arising from the injection site was visualized. If no afferent channel was identified, the first hot spot that appeared on the lymphoscintigraphic images was considered to represent the sentinel node. The location of a sentinel node was marked on the skin with ink.

The operation was performed the following day. After induction of general anaesthesia, 1·0 ml patent blue dye (Blue Patenté V; Laboratoire Guerbet, Aulnay-sous-Bois, France) was injected intradermally surrounding the tumour or biopsy site. Measurements were made over the skin mark with a γ ray detection probe (Neoprobe® 1000/1500; Neoprobe Corporation, Dublin, Ohio, USA) to confirm the location of the sentinel nodes seen on the scintigrams. Through an incision of a few centimetres, a blue-stained lymphatic channel was sought. Such a blue channel was dissected carefully to the site at which it drained into a lymph node. The probe was used to determine whether the blue node was radioactive. If no blue-stained lymphatic channel was found, the probe was used to guide the dissection. A lymph node was considered to be a sentinel node when a blue afferent lymphatic channel was identified coming from the injection site and leading to this node, or when the probe readings pointed to the same node that had been determined to be a sentinel node on the scintigraphy images.

All sentinel nodes were embedded completely for microscopic evaluation. Nodes up to 1 cm were processed bisected in one block while larger nodes were processed in parallel slices of 0·2 cm in several blocks. All blocks were cut at three levels at 100-μm intervals, and stained with haematoxylin and eosin and immunohistochemical techniques (S-100 and HMB-45). Regional node dissection was performed only if metastatic disease was found in a sentinel node. In the case of a tumour-containing sentinel node outside a lymphatic field, dissection of the subsequent nodal basin was performed.

Results

Hot spots outside the regular lymph basins were seen in 25 (6·6 per cent) of the 379 patients. During surgery, such a hot spot proved to be a lymphangioma and not a lymph node in two patients. One was situated on the upper back and the other on the thigh. A third patient's hot spot on the calf had disappeared after 24 h. The hot spot was not explored in four patients. In two of these patients, it was situated in the parasternal region, most likely in the internal mammary chain. The other two were seen in the mediastinum and in the infraclavicular fossa. Removing these hot spots was considered excessive in view of the experimental nature of the sentinel node procedure. Assuming that these four hot spots represented lymph nodes, sentinel nodes were seen at aberrant sites in 22 patients (5·8 per cent).

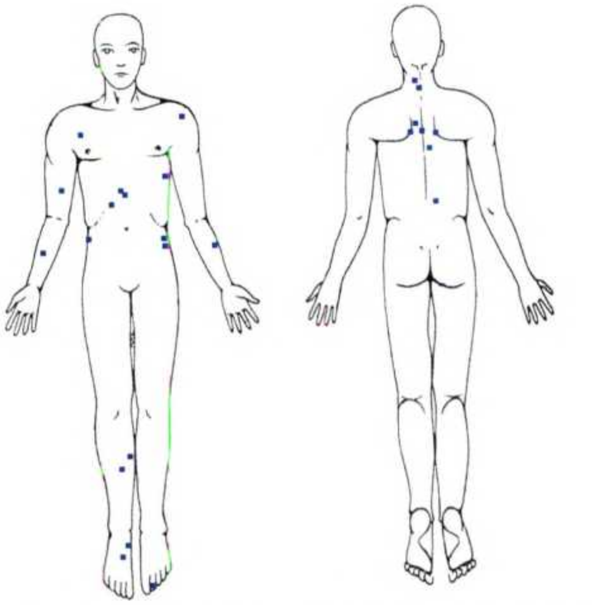

Fig. 1 Location of primary tumours draining to aberrant sentinel nodes

Radioactive sentinel lymph nodes were identified and harvested in the remaining 18 patients (4·7 per cent of the whole group). Eleven (8·3 per cent) of the 133 patients with a primary tumour on the trunk had an abnormally located sentinel node. For the arm and leg, these figures were four (7 per cent) of 58 and three (2·0 per cent) of 153 respectively. None of the 35 patients with a primary tumour in the head and neck region had an aberrant sentinel node.

The location of the primary tumours and the extra-regional sentinel nodes are presented in *Figs 1* and *2* respectively. Six patients with a melanoma on the trunk had sentinel nodes in the flank (four) or adjacent to the areola (two). Five patients had a melanoma on the upper back draining to sentinel nodes in the triangular intermuscular space. Three patients with a melanoma on the lower leg or foot had sentinel nodes in the popliteal fossa. Two sentinel nodes were found in the epitrochlear fossa, one over the deltoid muscle and one in the bicipital sulcus.

Four patients had metastasis in one of the aberrant lymph nodes and underwent dissection of the subsequent lymph node basin. The additionally removed lymph nodes were free of disease in three of these patients. In the fourth patient, a second sentinel lymph node was present in the subsequent lymph node basin.

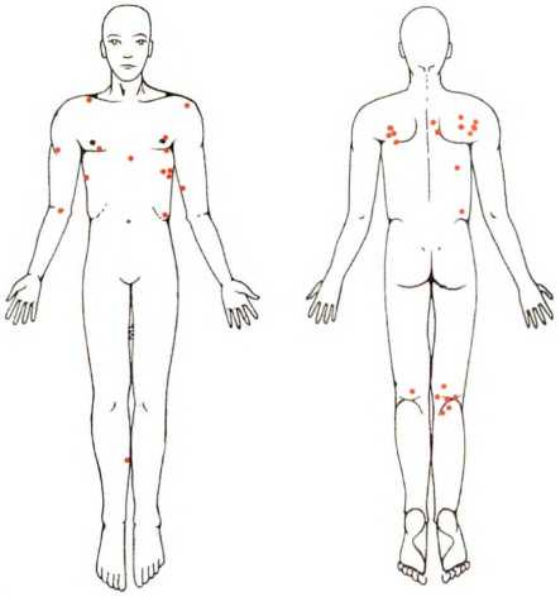

Fig. 2 Location of aberrant sentinel nodes

Discussion

Sentinel nodes outside regional lymph basins were seen in 22 (5·8 per cent) of the 379 patients and harvested in 18 (4·7 per cent). Specific drainage patterns were identified. Aberrant sentinel nodes would not have been noticed if only a blue dye or a probe had been used without lymphoscintigraphy. In five patients, the sentinel lymph nodes were found in the epitrochlear fossa (two) and the popliteal fossa (three). Although these fossae are known to harbour lymph nodes, they were included in the study because sentinel nodes are rarely found at these sites[2,3].

In five patients, sentinel nodes were found in the triangular intermuscular space. This entity is situated lateral to the scapula and surrounded by the supraspinatus muscle, infraspinatus muscle and the long head of the triceps muscle. The primary tumour was situated in between the scapulae in these cases. Uren *et al.*[4] have also described sentinel nodes in the triangular intermuscular space. Apparently, this is an existing drainage pattern. Until now, tumour spread at this location was probably regarded as in-transit metastasis rather than lymph node metastasis.

Two patients with a melanoma in the epigastric region had a sentinel node adjacent to the areola. After becoming aware of this possible lymph node location, two patients were seen who had not undergone sentinel node biopsy with an involved node at this specific site during follow-up. This is apparently an existing drainage pattern as well, although it has not been described in the literature.

In one patient a hot spot on the calf disappeared within 24 h. Possible explanations are contamination or temporary retention in a lymph vessel or lymphatic lake[5]. In two patients, a hot spot thought to be a sentinel node was removed during operation and proved to be a lymphangioma.

A possible explanation for an abnormal drainage pattern was found in a patient with a primary tumour on the right upper abdominal wall. One would expect the sentinel node to be in the right axilla. Previous removal of a cyst underneath the areola may have caused alternative drainage to sentinel nodes in the internal mammary chain and contralateral axilla.

In four (22 per cent) of 18 patients the extraregional sentinel nodes proved to be tumour positive. A similar proportion is found in sentinel nodes within regional lymph node basins[6–8]. In four patients the hot spot was not explored because it was situated at a site that was difficult to reach. Sentinel nodes may be situated in the internal mammary node chain, the mediastinum and the retroperitoneum[9–11]. Enlargement of lymph nodes in such locations is more likely in advanced disease compared with involvement of more superficially located lymph nodes. The fact that the primary tumours associated with such deep sentinel nodes are situated on the trunk may partially explain the poor prognosis of trunk melanomas compared with melanomas in the head and neck region and on the extremities.

An aberrant sentinel node may be the sole site of metastasis but may also indicate involvement of the subsequent lymph node basin. Pursuit of sentinel nodes outside nodal fields allows early removal of such additional metastases. Whether early regional node dissection leads to improved regional control and better survival is the subject of ongoing prospective randomized trials.

The incidence of aberrant sentinel nodes in this study was 5·8 per cent. Such nodes would not have been identified without lymphoscintigraphy. The majority were situated in the triangular intermuscular space, the flank and the popliteal fossa. Specific drainage patterns could be discerned. The percentage of tumour-positive sentinel nodes at aberrant sites was similar to that of sentinel nodes within a lymphatic field. An aberrant sentinel node may be the sole site of metastasis or may indicate involvement of the subsequent lymph node basin. Therefore, excision of these sentinel lymph nodes is recommended if lymphatic mapping becomes part of routine management.

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Standards of documentation of the surgeon–patient consultation in current surgical practice

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Background: This study was designed to assess the reliability of the system whereby junior doctors document the surgeon–patient consultation in general surgical practice in the UK.
Methods: A prospective observational study was carried out, with an independent observer recording details of the surgeon–patient consultation and assessing the reliability of documentation. An exact record of the information given by consultants was transcribed in shorthand at the time of consultation. Data were recorded in the categories recommended for notation by the General Medical Council: clinical findings, decisions made, information given to patient and treatment prescribed. Case notes were examined within 24 h of the ward round to ascertain reliability of the documentation.
Results: The study population comprised 432 surgeon–patient consultations. There were important deficiencies in the documentation of consultants' clinical findings and management decisions. In addition, information given by consultants to the patients regarding clinical findings and treatment planned (including the need for operation) was recorded in a median of 6 per cent of consultations.
Conclusion: This study provides objective evidence of shortfalls in the documentation of the surgeon–patient consultation process. These deficiencies are such that, under present circumstances, the requirements of the General Medical Council with respect to case note documentation are not fulfilled in this setting.

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Introduction

Current guidelines for good medical practice issued by the General Medical Council[1] state that every doctor should 'keep clear, accurate and contemporaneous patient records which report the relevant clinical findings, the decisions made, the information given to patients and any drugs or other treatment prescribed'. In the context of general surgical practice it may be possible to adhere to these guidelines in the outpatient setting, but for inpatients the daily record of the consultant ward round is often made by junior doctors. This note may be the only contemporaneous record of the process of care. To date, there are no objective data to assess the reliability with which these consultations are documented. This study examined the reliability with which the care plan formulated by the consultant is recorded in order to ascertain the validity of this system of documentation of the care process in contemporary surgical practice.

Patients and methods

Study design

Data were recorded prospectively on patients admitted to the general surgical unit of a university teaching hospital over an 8-week study period. The unit comprised a hepatobiliary and upper gastrointestinal service, a colorectal service and a general surgical emergency service. Each morning a consultant ward round from one of these services was selected for study (the three services being sampled with equal frequency) and data pertaining to the consultant–patient consultation were recorded by an independent observer (K.J.F.). An exact record of the information given by consultants was transcribed in shorthand on to a notepad by the observer at the time of each doctor–patient consultation. The observer had no involvement in the process of delivery of care. Data were recorded in the categories recommended for notation by the General

Medical Council[1]. These were: clinical findings, decisions made, information given to patient and treatment prescribed. Case notes were examined within 24 h of the ward round (but after the end of the normal working day at 17.00 hours) to ascertain what proportion of the consultants' recommendations had been documented.

All 11 consultant surgeons participating in the acute general surgery urgency rota were included and no comparisons were made between individuals. All participating consultants accepted that they would not be notified of the dates of commencement and completion of the study or the dates when their ward rounds would be sampled. All data are expressed as median (range).

Assessments for bias

During a preliminary validation period the accuracy of the observer's shorthand notation was compared with the recommendations given by an individual consultant (A.K.S.). This validation period confirmed that all key information points were transcribed consistently. The results obtained during this validation period were excluded from the present study. After completion of the study, results obtained in the first 4 weeks were compared with those obtained in the second half of the study to check for changes in practice resulting from the presence of the observer. The proportions of responses documented in all categories were similar in both time periods (data not shown).

Ethical approval

The study was approved by the Lothian ethics committee and by the institutional research ethics committee of the Royal Infirmary of Edinburgh.

Results

The study population comprised 432 consultant–patient consultations arising from 35 consultant ward rounds. During the period of this study the results of clinical consultation were recorded by the consultant in person on three occasions. These entries were excluded. Documentation of information by specialist registrars (three) was also excluded.

Documentation of clinical findings

Recognition by the consultant of the presence of gastrointestinal symptoms such as abdominal pain, nausea or vomiting was recorded on 63, 53 and 38 per cent of occasions respectively (*Table 1*). Findings of abdominal examination were recorded in a median of 77 (range 33–100) per cent of instances. Findings of immediate clinical significance such as abdominal tenderness and signs of peritonitis were recorded on 52 (91 per cent) of 57 and on two of two occasions respectively. Observations made by consultants with respect to charts and diagnostic tests were recorded on a median of 62 per cent of occasions.

Table 1 Clinical findings

	No. of consultant findings	No. of findings recorded by house officers	Findings documented (%)
Gastrointestinal symptoms			
General well-being	63	42	67
Abdominal pain	51	32	63
Nausea	15	8	53
Vomiting	32	12	38
Passage of flatus	14	5	36
Bowel movements	20	5	25
Diarrhoea	10	6	60
Weight loss	7	0	0
Other	23	14	61
Median of findings documented			45
Abdominal examination			
Abdominal tenderness	57	52	91
Abdominal distension	5	4	80
Abdominal masses	6	2	33
Signs of peritonitis	2	2	100
Bowel sounds	15	11	73
Jaundice	6	3	50
Abdominal surgical wound	18	10	56
Contents of drain	24	14	58
Rectal examination	1	1	100
Other	8	7	88
Median of findings documented			77
Review of charts and diagnostic tests			
Vital signs	64	47	73
Fluid balance	7	5	71
Drain output	34	20	59
Review of blood results	34	18	53
Radiology results	16	10	62
Median of findings documented			62

Documentation of decisions

A median of 74 (range 58–100) per cent of consultant recommendations were documented (*Table 2*). However, decisions on surgically important aspects of care such as adjustment or removal of abdominal drains were recorded in only 59 per cent of consultations. Similarly, the decision to discharge the patient from hospital was not recorded in 16 per cent and no record was made of consultants' plans for outpatient follow-up in 29 per cent of cases. Documentation of investigations recommended by consultants took place on a median of 87 (range 67–100) per

Table 2 Decisions made

	No. of consultant recommendations	No. of recommendations recorded by house officers	Recommendations documented (%)
Clinical progress			
Change in intravenous fluid intake	18	12	67
Change in oral fluid intake	73	52	71
Change in diet	66	53	80
Change in parenteral feed	6	6	100
Abdominal drains	29	17	59
Nasogastric tube	8	6	75
Urinary catheter	15	11	73
Removal of clips	12	7	58
Removal of central venous line	8	6	75
In-hospital referral	58	46	79
Outpatient follow-up	24	17	71
Discharge from hospital	74	62	84
Median of decisions documented			74
Investigations recommended			
Blood tests	91	66	73
Radiography	15	13	87
Computed tomography	23	20	87
ERCP	24	24	100
Ultrasonography	49	44	90
Tubography	11	9	82
Laparoscopic ultrasonography	8	7	88
Gastrografin swallow	9	8	89
Gastrografin enema	4	4	100
Barium enema	9	8	89
Barium meal	3	2	67
Endoscopy	10	8	80
Other	46	32	70
Median of decisions documented			87

ERCP, endoscopic retrograde cholangiopancreatography

cent of occasions. Again, 20 per cent of recommendations for endoscopy were unrecorded, as were 33 per cent of requests for barium meals.

Documentation of information given to patients

Documentation of information given by consultants to patients took place on a median of 6 (range 0–11) per cent of occasions (*Table 3*). Three of 27 explanations to patients that operation was required were documented.

Documentation of treatment prescribed

Prescription of analgesia was recorded in 19 of 25 instances and prescription or change of antibiotics was recorded in 38 of 48 cases.

Table 3 Information given to patients

	No. of consultant recommendations	No. of recommendations recorded by house officers	Recommendations documented (%)
Clinical findings	62	7	11
Decisions made	118	2	2
Investigations	22	1	5
Treatment prescribed	12	0	0
Operations required	27	3	11
Miscellaneous	13	1	8
Median of discussion points documented			6

Discussion

This prospective observational study has highlighted apparent shortcomings in the documentation of the process of inpatient care in a general surgical service. Interpretation of any such deficiencies must take into consideration the limitations inherent in this type of observational study. It is possible that junior staff may have gained awareness of the study in progress, thus resulting in artificially raised standards of documentation. While it was not possible to control for this risk, comparison of the proportion of findings documented in the second 4 weeks to those documented in the first 4 weeks showed no difference in patterns of documentation between the two time periods. It could be argued that examination of the case notes within 24 h of the consultant ward round allowed insufficient time for documentation of information. However, the purpose of continuation notes is to provide a daily record of ongoing care. Entries not documented within 24 h of the consultation are unlikely to be regarded as contemporaneous. Further, if information was not recorded by the end of the day, the next entry in the notes may well pertain to the subsequent day. Finally, the patterns of case note documentation may be biased by idiosyncrasies in a single ward or group of junior doctors. As this study sampled a range of general surgical services over a range of wards and a wide group of junior doctors, such a possibility seems unlikely.

Care must also be exercised in extrapolating the findings of this study. While it is possible that these findings may represent local problems within a single institution or service, there is no reason to suspect that these problems are not more widespread in general surgical services throughout the UK. If other markers of clinical outcome are examined as indirect indicators of the quality of care provided by the general surgical service in the present study, favourable results have been reported in patients undergoing major surgery such as hepatic resection[2], Whipple pancreaticoduodenectomy[3] and colorectal cancer resection[4]. There are thus no pointers to suggest that apparent deficiencies in documentation are a reflection of poor-quality care.

The results suggest that there may be appreciable underdocumentation of the consultant–patient consultation in all categories. In terms of symptoms and physical examination, appreciable proportions of consultant findings were not documented. In terms of reflecting the process of care, the limited documentation of practical treatment decisions has potentially far-reaching consequences. These deficiencies extend to important therapeutic decisions such as prescribing analgesia and antibiotics.

Information given to patients was uniformly poorly documented. The medicolegal implications of this deficiency in documenting information may be considerable. Although the consultant may have provided detailed information to the patient, the lack of documentation severely compromises any subsequent need to justify or defend the decision-making process.

Solutions to the problems highlighted by this study are not straightforward. Given the current levels of workload for consultant surgeons, it does not seem feasible to suggest that all consultants should document findings by the bedside for every patient. A compromise solution consisting of documenting only major decisions or those likely to be challenged subsequently seems unsatisfactory. It is not always possible to predict the situations and clinical decisions that may later be deemed to be in need of justification. The use of modern technological aids may provide a solution. For example, hand-held dictating machines may improve the accuracy of transcription of information but will increase demands on secretarial support. Greater application of voice recognition software may also be of value.

In conclusion, this study has demonstrated appreciable shortfalls in the documentation of the consultant–patient consultation process within a general surgical service. Under present circumstances, the requirements of the General Medical Council with respect to case note documentation are not fulfilled and may not be feasible within the context of inpatient general surgical practice.

Acknowledgements

The authors acknowledge the support of the consultant staff of the surgical corridor of the Royal Infirmary of Edinburgh.

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Correspondence

The Editors welcome topical correspondence from readers relating to articles published in the Journal. Letters should be no more than 250 words in length and should be typed on A4-sized paper in double spacing

Instant Anatomy. 2nd ed. (*Br J Surg* 2000; 87: 1254)

Sir

I write regarding comments attributed to S. Nicholson in his book review in which it is stated that anatomical eponyms exist 'as a source of amusement to elderly anatomists'. Over the years the use of eponyms has been condemned by many people, yet the very fabric of medicine is woven from a rich and vibrant history. The use of eponyms intimates at this and behind each one there usually lies an insight into the personalities and practices of a bygone medical era. To deny the value of eponyms is to deny the very essence of our medical heritage and for the record I am neither elderly or an anatomist!

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Authors' reply

Sir

Dr Washington clearly mourns the passing of a gentler era. As a basic surgical trainee he will soon learn, if he has not already, that the modern day trainee must assimilate a huge amount of highly technical knowledge. There simply is not the time to concentrate on fascinating historical vignettes. Modern surgical anatomy must be learned and understood in functional and practical terms integrated with a knowledge of physiology and pathology. Eponymous descriptions simply serve to distract from the important issues. While they may remain fascinating for the anatomical anoraks they are not relevant today.

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Multicentre study of thyroid nodules in patients with Graves' disease (*Br J Surg* 2000; 87: 1111-13)

Sir

We recently analysed our data of 130 patients with surgically managed Graves' disease to examine the implications of thyroid nodules in an endemically iodine deficient area. The relevant findings were: (1) thyroid nodules were observed in 30 per cent of patients with Graves' disease, comparable to the findings of the authors, though we expected a higher incidence in an iodine deficient area. However, 86 per cent of nodules were palpable before operation and the rest were detected peroperatively. As we do not use ultrasonography for evaluation of these patients, the true incidence may be much higher; (2) five patients had associated carcinoma (four women and one man). The median age of these patients was 45 years. The overall incidence of carcinoma in Graves' disease was 6 per cent while in patients having nodules it was 14 per cent; (3) histology was papillary in four patients and follicular carcinoma in one. The median tumour diameter was 10 mm. Two patients had multicentric carcinoma and one had cervical node involvement.

In our earlier study we found that presence of thyroid nodules in Graves' disease is a predictor of high incidence of carcinoma[1]. Therefore we perform total thyroidectomy as a protocol in these cases. We support the authors' view that these patients should be advised to have early surgery and the ideal operation should be total thyroidectomy.

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1 Thakur S, Sharma AK, Agarwal A, Mishra SK, Bhatia E. Carcinoma in Graves' disease. *J Assoc Physicians India* 1995, 43: 600-1.

Prospective analysis of a scoring system to predict choledocholithiasis (*Br J Surg* 2000; 87: 1176-81)

Letter 1

Sir

I read with interest the article by Menezes *et al.* Of the factors that were retrospectively studied, both jaundice and bilirubin levels were analysed for their association with common bile duct (CBD) stones. Jaundice is a clinical manifestation of a raised bilirubin level. Analysing them separately for an association with CBD stones and suggesting that 'the significance of increased serum bilirubin levels was lost in the multivariate analysis. This may be due to the close association between serum bilirubin concentration and jaundice, leading to the exclusion of the former, as is a feature of multivariate analysis' is therefore not appropriate. Jaundice is evident when the serum bilirubin rises above 34 mmol/l. Jaundice and high bilirubin levels are not closely associated; they are one and the same.

In the prospective part of the study, the incidence of CBD stones has been compared in patients with a score of 3 or more (42 per cent) and in patients who scored less than 3 (4 per cent), but the time of definite diagnosis of CBD stones in the two arms are different (endoscopic retrograde cholangiopancreatography (ERCP) within 6 weeks and elective cholecystectomy within 3 months). The authors state that 'stones migrate fairly rapidly out of the CBD during or soon after the acute episode, and this may explain a detection rate of less than 50 per cent in this group of patients as the time interval between the scoring of patients and ERCP may have been as long as 6 weeks'. The time interval between scoring of patients and elective cholecystectomy has been as long as 3 months.

Another point of note is that certain factors studied retrospectively for their association with CBD stones (ascending cholangitis, dilated CBD and CBD stone on ultrasonography) are by themselves definite indications for ERCP before a laparoscopic cholecystectomy and may not really merit further study.

Lastly, what factors were present in the five patients who had a score of less than 3 but still had CBD stones? I would strongly suspect that they had a moderately raised bilirubin (not enough to produce jaundice) and a high alkaline phosphatase. Though these two factors do not figure in the scoring system, I think they would be enough to persuade many surgeons to perform an ERCP before a laparoscopic cholecystectomy.

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Letter 2

Sir

Menezes *et al.* state that performing ERCP on patients suspected of having CBD lithiasis is a common policy. However, in a single-session, laparoscopic cholangiography or ultrasonography allows the diagnosis of CBD stones and then treatment by either laparoscopy or converted laparotomy. This strategy is safe and effective[1–3] and a European prospective randomized study suggests that preoperative ERCP and sphincterotomy should be reserved for poor risk patients[4].

In their multivariate study for predicting choledocholithiasis, the inclusion of covariables such as jaundice or CBD stone on preoperative ultrasonography are not relevant as, in these patients, the CBD has to be explored.

The incidence of CBD stones was unusually high (33 per cent) when compared with the reported incidence of 10 per cent in Western countries. Therefore, routine operative exploration of the CBD is unnecessary in 90 per cent of patients. Consequently, one cost-effective policy is to identify a subgroup of patients having a very low risk of CBD stones thereby avoiding exploration of the CBD. The negative predictive value of a score seems to be more useful than a positive predictive value. In our multivariate study[5] of 566 patients operated on for gallbladder lithiasis without preoperative evidence of CBD stones, the significant predictors of CBD stones were a CBD diameter of greater than 12 mm (relative risk (RR) 22·0), gallbladder stones less than 10 mm (RR 3·3), age greater than 75 years (RR 5·6), cholecystitis (RR 2·0), and previous history of biliary colic (RR 2·7). A scoring system was determined to produce the best cut-off point to predict a low probability of CBD stones. For a score of less than 3·5, this probability rate was less than 2 per cent. In our series, if CBD exploration had been used selectively, it would have avoided exploration in 46 per cent of patients. However, CBD stones would have been missed in five patients. This scoring system has been validated in two other groups of patients[6,7].

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Authors' reply

Sir

It is worth re-emphasising that the incidence of CBD calculi in the patients undergoing gallbladder surgery in this study was 15 per cent. The point made by Mr Balasubramanian relating to the incidence of CBD calculi at preoperative ERCP (42 per cent) simply illustrates the dynamic nature of this study of patients undergoing gallbladder surgery over a time period as commonly experienced in the UK. While constructing a scoring system to selectively choose patients for a preoperative ERCP, it is important to include all relevant clinical, biochemical and ultrasound criteria. Exclusion of important factors would decrease the sensitivity of the scoring system.

Despite suspicion regarding the five patients who had a score of less than 3 we repeat that none of these patients had deranged liver function tests.

We agree that a single-session laparoscopic procedure for gallbladder and choledocholithiasis is entirely acceptable, but we have no personal experience of this approach. We congratulate Dr Hugier for constructing a scoring system comparable with our own study.

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Prospective randomized trial of low-pressure pneumoperitoneum for reduction of shoulder-tip pain following laparoscopy (*Br J Surg* 2000; 87: 1161-5)

Sir

I found the study conducted by Sarli *et al.* interesting, but there are certain flaws. It hypothesises that reduced stretching of the diaphragmatic peritoneum helps to reduce shoulder-tip pain. If this mechanism were true, one would surely expect bilateral shoulder-tip pain as both sides of the diaphragm are equally stretched. The paper notes that reducing the pneumoperitoneum pressure reduced the frequency and intensity of shoulder-tip pain. However, despite the efforts made it is difficult to differentiate shoulder-tip pain from abdominal pain when determining the need for analgesia, and analgesic consumption is only quoted for the first 24 h after operation. Was the total analgesic consumption in the two groups the same?

The trial was established to look at analgesia requirements, not intra- or postoperative complications. It is impossible to state from this study that there is no increased risk from using low-pressure pneumoperitoneum, for example through decreased visibility, and until this question is answered this technique cannot be advocated. In this regard we note that four patients were withdrawn as laparoscopy was converted to an open procedure, but we are not told the reason for conversion.

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Authors' reply

Sir

We agree that one would expect that stretching the diaphragmatic peritoneum should cause bilateral shoulder-tip pain but the aetiology of this pain is complex and several factors are involved. Subdiaphragmatic entrapment of bubbles of carbon dioxide[1] and the raw area of the removed organ[2] are at least partially responsible for shoulder pain after laparoscopic cholecystectomy and this may explain the higher frequency of shoulder-tip pain on the right side. Most of the reports are based on an *ex juvantibus* criterion. By demonstrating that a reduction in carbon dioxide pressure is associated with a reduced frequency and intensity of shoulder-tip pain, this allows a supposition that stretching of the diaphragmatic peritoneum is an important co-factor in causing postoperative pain, as proposed by Wallace *et al.*[3]. The elimination of one of the co-factors is sufficient to significantly reduce the frequency and intensity of the pain. Low-pressure pneumoperitoneum was useful in reducing postoperative intravenous analgesic requirements but not oral analgesic consumption after discharge, which was very low in both groups. We did not observe any intra- or postoperative complications during the study. The only conversion to an open procedure in the low-pressure pneumoperitoneum group was due to the presence of intra-abdominal adhesions.

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1 Jorgensen JO, Gillies RB, Hunt DR, Caplehorn JR, Lumley T. A simple and effective way to reduce postoperative pain after laparoscopic cholecystectomy. *Aust N Z J Surg* 1995; 65. 466-9.
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Surgical and Endovascular Treatment of Aortic Aneurysms

A. Branchereau, M. Jacobs (eds)

277 × 215 mm. Pp 315 Illustrated. 2000 Armonk Futura $125·00.

This is the first textbook of the 21st century dedicated to the subject of surgical and endovascular treatment of aortic aneurysms. It addresses a broad spectrum of issues, including epidemiology and natural history, quality of life, and safety regulations concerning new techniques. The book was designed to accompany the European Vascular Course held in Marseilles in 2000 and includes chapters from each of the authors invited to speak at this vascular symposium.

The subject matter covered is extremely concise and the author list includes many of the great and good who practise both vascular surgery and vascular radiology within the Western world. These authors are drawn from as far afield as Los Angeles, Rio de Janeiro and Sydney; nevertheless, the book is primarily European in style and the subject matter covered is bang up-to-date. Many of the early chapters, especially those on epidemiology and natural history, have been reproduced previously, but the book does contain many new concepts, such as the classification of endovascular procedures, and concentrates as much on thoracic as it does on abdominal aortic aneurysms. There is a strong section describing the complications of endovascular aneurysm repair and the various registries are covered in detail. I was particularly fascinated by Michael Jacob's chapter concerning monitoring of spinal cord ischaemia during thoracoabdominal aneurysm repair and Hazim Safi's chapter detailing circulatory support during the same procedure. The results of thoracic aneurysm repair are given in detail by Edward Kieffer and the book contains equally impressive chapters on infrarenal aortic aneurysm repair in high-risk patients and techniques involved in the treatment of juxtarenal aortic aneurysms. Perhaps the most challenging chapters concern new techniques for the repair of abdominal aortic aneurysms. These include laparoscopic resection and video-assisted aortic surgery. Results are scanty but I suspect that both techniques will become popular within the next 5 years.

In summary, this volume is a valuable aid to any vascular specialist performing both straightforward and complex aortic aneurysm surgery. Each of the authors is an expert in their particular field, but it must be appreciated that the views expressed are international and are not always a reflection of local practice. This book is essential reading for any vascular interventionist wishing to gain an insight into how the experts manage complex aortic aneurysms.

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Neoplasms of the Colon, Rectum and Anus

P. H. Gordon, S. Nivatvongs

285 × 222 mm Pp. 368. Illustrated. 2000. St Louis Quality Medical Publishing. $110·00.

This is a good-sized, attractive book divided into seven chapters. The layout is user-friendly, the text makes easy reading and each chapter has a well balanced bibliography. The authors are based in Canada and the USA. Chapter 1 deals with benign neoplasms of the colon. While advocating colonoscopy, the authors state that 'most polyps throughout the entire colon and rectum can be excised through the colonoscope with minimal morbidity'. I take this to mean that most polyps can be excised using the colonoscope rather than through the scope. The clear message from chapter 1 is that after the colon and rectum are clear of polyps, repeat colonoscopy should be performed every 3-5 years. Chapter 2 covers colorectal cancer screening, surveillance and follow-up. The general aim is to try to reduce the Dukes' staging of the tumour by the time of presentation and diagnosis. The new UK ionizing radiation (medical exposure) regulations (13 May 2000) require that radiographic investigations be carried out only after appropriate justification procedures. Barium enema and colonoscopy are therefore not interchangeable and UK trainees must be aware of the increasing trend towards more colonoscopies. Chapter 3 covers malignant neoplasm of the colon and describes the incidence and projected death rates in the USA and Canada. Surgical techniques of stapling and suturing and subsequent adjuvant therapy are well covered. Chapter 4 covers malignant neoplasms of the rectum with a particularly good discussion of lymph node excision. Management of sacral and perineal defects with use of rectus abdominis is covered, although I prefer the gluteus maximus transpositions. Chapter 5 covers perianal and anal neoplasm, both surgical and non-surgical approaches. The identification of anatomical landmarks is stressed. Chapter 6 covers transanal microsurgery (TEM), not to be confused with total mesorectal excision (TME) for carcinoma of the rectum. Chapter 7 covers laparoscopic colorectal surgery. I agree that the Veress needle is an unnecessary risk at the beginning of the operation. Left colonic surgery is described as a laparoscopic version of the open procedure. I think that the European method described by Marescaux is a slightly better approach. With this technique the colon is initially left attached to the lateral side of the abdomen while the vessels are identified on the medial side through the mesentery. In summary, this is an excellent book with good illustrations. I can recommend it for training and for general and colorectal surgeons.

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Minimal Access Surgical Anatomy

C. E. H. Scott-Conner, A. Cuschieri, F. J. Carter

285 × 220 mm. Pp 305 Illustrated. 2000. Philadelphia. Lippincott Williams and Wilkins. $125·00

There is probably no surgical procedure within the abdomen or retroperitoneum that has not been performed by the laparoscopic approach. Knowledge of anatomy is the fundamental tool for any successful operation. Although anatomical principles and details do not change in minimal access surgery, there is a different perspective for the surgeon who is faced closely with organs, spaces and dissecting planes. The authors have realized this special aspect when preparing *Minimal Access Surgical Anatomy*. Eleven chapters are organized according to topographical regions and organs. They cover the anterior abdominal wall, the peritoneal cavity, the diaphragm, the hiatus and the whole gastrointestinal tract, as well as the hepatobiliary system, the spleen and pancreas, the kidneys and adrenal glands, the inguinal region and the pelvis. Each chapter is introduced by a text that totals 62 pages. The text is followed by an extensive section of illustrations, with 220 colour photographs from laparoscopic videotapes. Every photo is accompanied by a legend and a sketch, explaining the figures in detail. Unfortunately some photographs are of very poor quality and so do not clarify the anatomy well; the main reasons are unrealistic colour and a lack of sharpness. The authors are well known experts in their fields: minimal access surgery, teaching and surgical anatomy. They have succeeded in integrating anatomy into the teaching of minimal access surgery. Regrettably some illustrations (an important part of an atlas) are not as perfect as they should be in a guide for the practising surgeon.

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Textbook of Prostatitis

J. C. Nickel (ed.)

282 × 222 mm Pp. 378. Illustrated 1999 Oxford Isis Medical Media £89·95

In his final chapter the editor claims. 'This textbook of prostatitis is the first comprehensive text on prostatitis and provides the clinician, the researcher, and even the interested patient with an authoritative resource on a very difficult clinical problem'. One cannot dispute this claim: this book is beautifully produced with superb illustrations and has an excellent clarity of presentation. There is a long list of contributors from around the world but most are North American; all are recognized experts in this field. I particularly enjoyed the chapters on the microbiological aspects of prostatitis and on seminal vesiculitis. However, this textbook contains greater detail than will be desired or required by the average clinician, and I found the text very repetitive, particularly that relating to the classification of prostatitis and to techniques of specimen collection and culture. Perhaps a little tighter editing of the contributions might have eliminated this problem. While acknowledging that this subject suffers from a paucity of hard clinical data, a more didactic approach by the authors would further help to make this a more attractive offering for the clinician. Undoubtedly this volume fills a gap in the market by reason of its comprehensive approach to the subject, but in an era of information overload this reader would have preferred a more concise offering.

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Scientific Surgery

Velmahos GC, Demetriades D, Shoemaker WC, Chan LS, Tatevossian R, Wo CCJ *et al.* Endpoints of resuscitation of critically injured patients: normal or supranormal? A prospective randomized trial. *Ann Surg* 2000; **232**: 409–18.
Some 75 patients with shock due to bleeding were initially resuscitated to normal or optimal physiological values. Patients who achieved optimal values in both groups did best (no deaths), but there was no significant difference in outcome between the two randomized groups (mortality rate 15 per cent in optimized patients *versus* 11 per cent in controls).

Yeo CJ, Cameron JL, Lillemoe KD, Sauter PK, Coleman J, Sohn TA *et al.* Does prophylactic octreotide decrease the rates of pancreatic fistula and other complications after pancreaticoduodenectomy? Results of a prospective randomized placebo-controlled trial. *Ann Surg* 2000; **232**: 419–29.
Some 211 patients were included; overall complication rates (40 per cent octreotide and 34 per cent controls) and pancreatic fistula rates (11 and 9 per cent, respectively) were similar

Taylor E, Dev V, Shah D, Festekjian J, Gaw F. Complicated appendicitis: is there a minimum intravenous antibiotic requirement? A prospective randomized trial. *Am Surg* 2000; **66**: 887–90.
Ninety-four patients were randomized to receive a minimum of 5 days antibiotics or a course of antibiotics discontinued on the basis of clinical factors There was no difference in the rate of infective complications or the duration of hospital stay, but the former group received more antibiotics.

Derzie AJ, Silvestri F, Liriano E, Benotti P. Wound closure technique and acute wound complications in gastric surgery for morbid obesity: a prospective randomized trial. *J Am Coll Surg* 2000; **191**: 238–43.
Some 331 patients were randomized to continuous or interrupted fascial closure Continuous closure resulted in fewer total complications (18 *versus* 31, $P = 0.02$) and fewer deep wound complications (5 *versus* 22, $P = 0.003$)

Camma C, Giunta M, Fiorica F, Pagliaro L, Craxi A, Cottone M. Preoperative radiotherapy for resectable rectal cancer: a meta-analysis. *JAMA* 2000; **284**. 1008–15.
All randomized controlled trials published in the last 30 years were analysed. Advantages of radiotherapy were relatively small but included reduced 5-year mortality rate (odds ratio 0·84, 95 per cent confidence intervals 0·72–0 98) and local recurrence rate (0·49; 0 38–0 62).

Richard CS, Gregoire R, Plewes EA, Silverman R, Burul C, Buie D *et al.* Internal sphincterotomy is superior to topical nitroglycerin in the treatment of chronic anal fissure. *Dis Colon Rectum* 2000; **43**: 1048–58.
Sphincterotomy significantly improved healing rate at 6 weeks (89 *versus* 29 per cent) and reduced the requirement for further surgery (3 *versus* 45 per cent) in this trial including 82 patients.

Carapeti EA, Kamm MA, Nicholls RJ, Phillips RKS. Randomized, controlled trial of topical phenylephrine for fecal incontinence in patients after ileoanal pouch construction. *Dis Colon Rectum* 2000; **43**. 1059–63.
This was a crossover study of 12 patients; six improved with phenylephrine (four had no incontinence), compared to one control. Phenylephrine significantly improved continence scores and resting anal pressures

Gioffre Florio MA, Bartolotta M, Miceli JC, Giacobbe G, Saitta FP, Paparo MT *et al.* Simple *versus* double jejunal pouch for reconstruction after total gastrectomy. *Am J Surg* 2000; **180**: 24–8.
Eighteen patients had a single, and 23 a double pouch. Nutritional recovery, weight, patient satisfaction and quality of life were all improved by the double pouch method.

Cohn-Cedermark G, Rutqvist LE, Andersson R, Breivald M, Ingvar C, Johansson H *et al.* Long-term results of a randomized study by the Swedish Melanoma Study Group on 2 cm *versus* 5 cm resection margins for patients with cutaneous melanoma with a tumour thickness of 0·8–2·0 mm. *Cancer* 2000; **89**: 1495–1501.
Median follow-up was 11 years in the 989 patients initially randomized; 146 (15 per cent died from their melanoma). There was no difference in survival or rate of local recurrence between the two resection margins.

Patterson EJ, Herron DM, Hansen PD, Ramzi N, Standage BA, Swanstrom LL. Effect of an esophageal bougie on the incidence of dysphagia following Nissen fundoplication. A prospective, blinded, randomized clinical trial. *Arch Surg* 2000; **135**: 1055–62.
The rate of late dysphagia was reduced from 31 to 17 per cent by the use of a 56F bougie ($P = 0\ 05$) in this study involving 171 patients No patient died but there was one significant bougie injury

Satlz LB, Cox JV, Blanke C, Rosen LS, Fehrenbacher L, Moore MJ *et al.* Irinotecan plus fluorouracil and leucovorin for metastatic colorectal cancer. *N Engl J Med* 2000; **343**: 905–14.
Some 683 patients were randomly allocated one of three different chemotherapy regimens. The regimen including weekly irinotecan, fluorouracil and leucovorin produced the best disease-free and overall survival.

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On the title page give: (1) the title of the article, (2) the name and initials of each author, (3) the department and institution to which the work should be attributed; (4) **the name, postal and email addresses, telephone and facsimile numbers of the author responsible for correspondence and to whom requests for reprints should be addressed;** (5) sources of financial support; and (6) the category in which the manuscript is being submitted (original article, review, etc).

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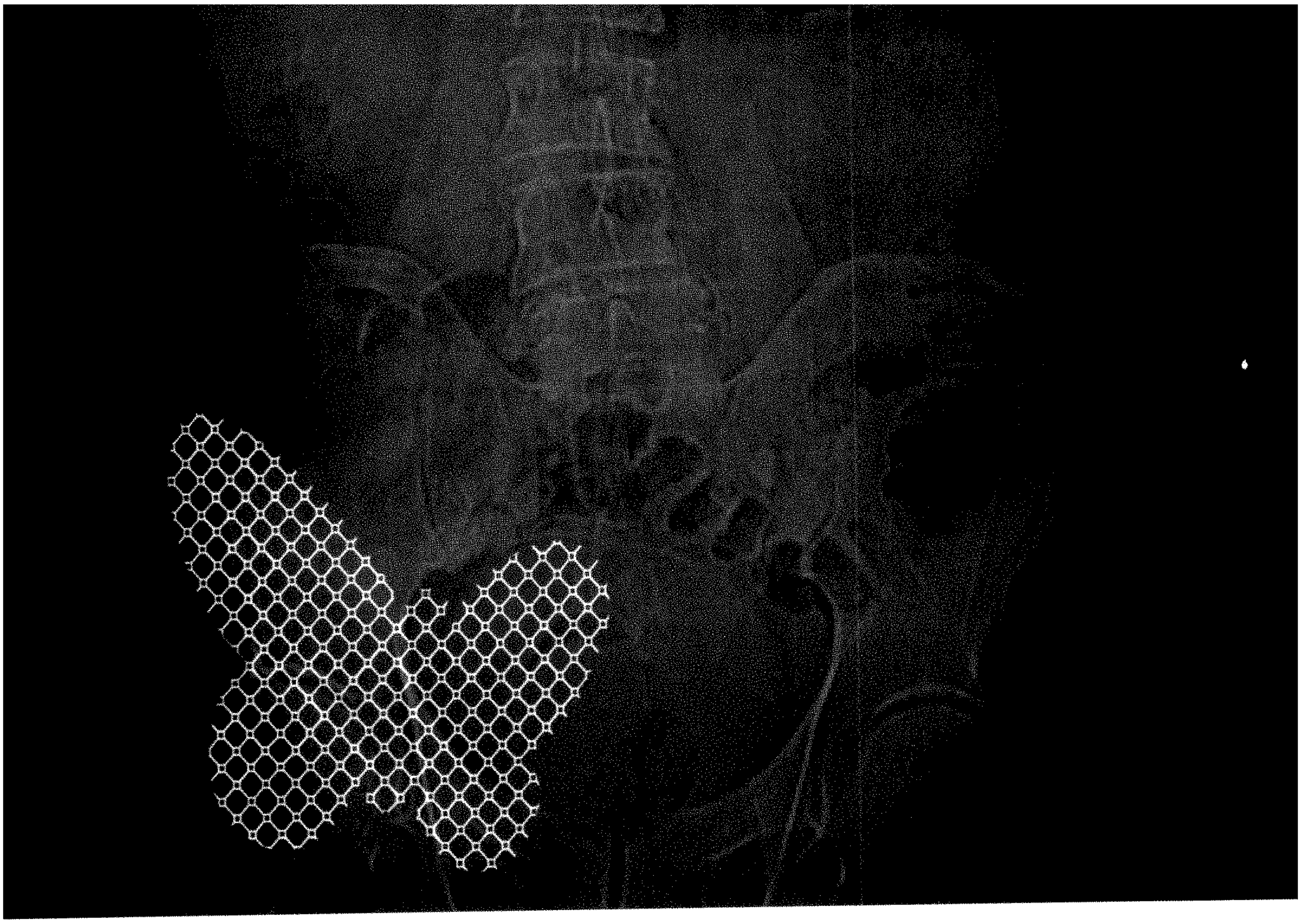

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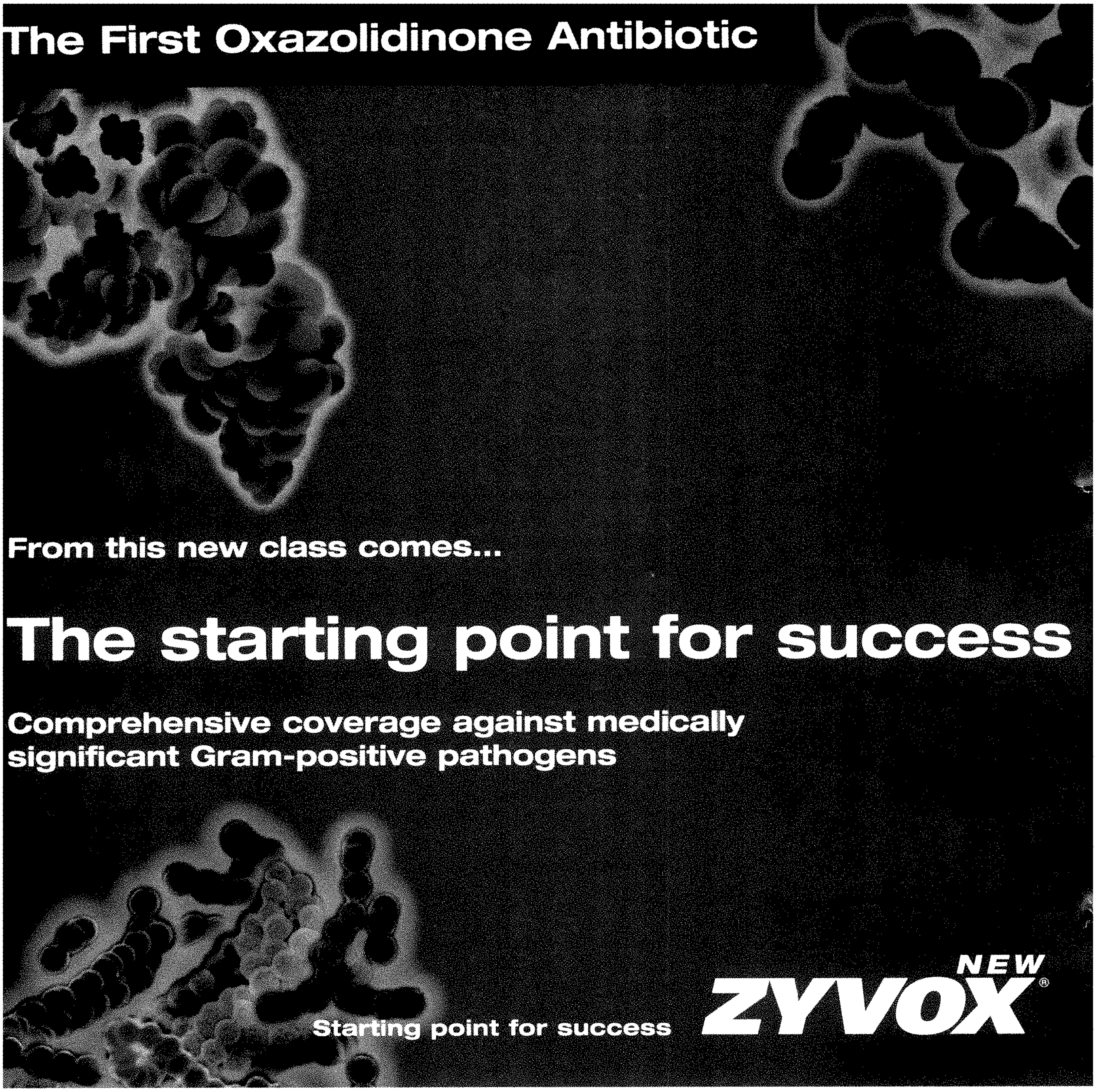
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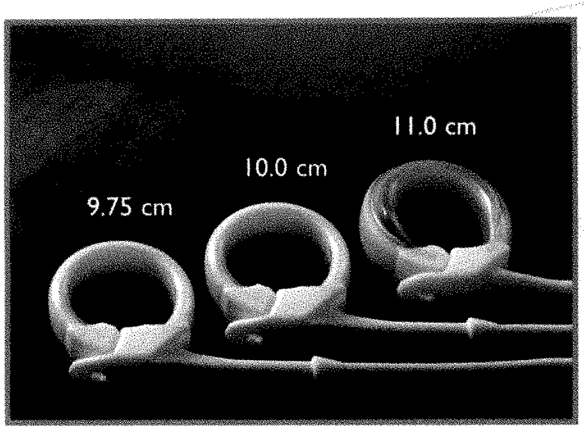

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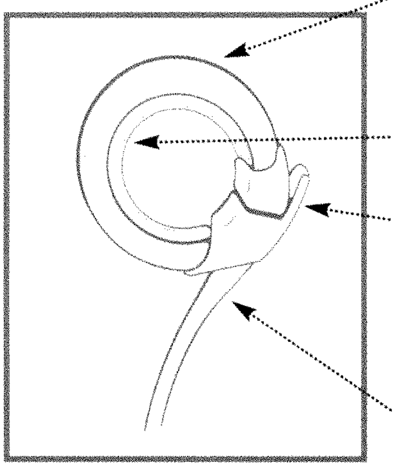

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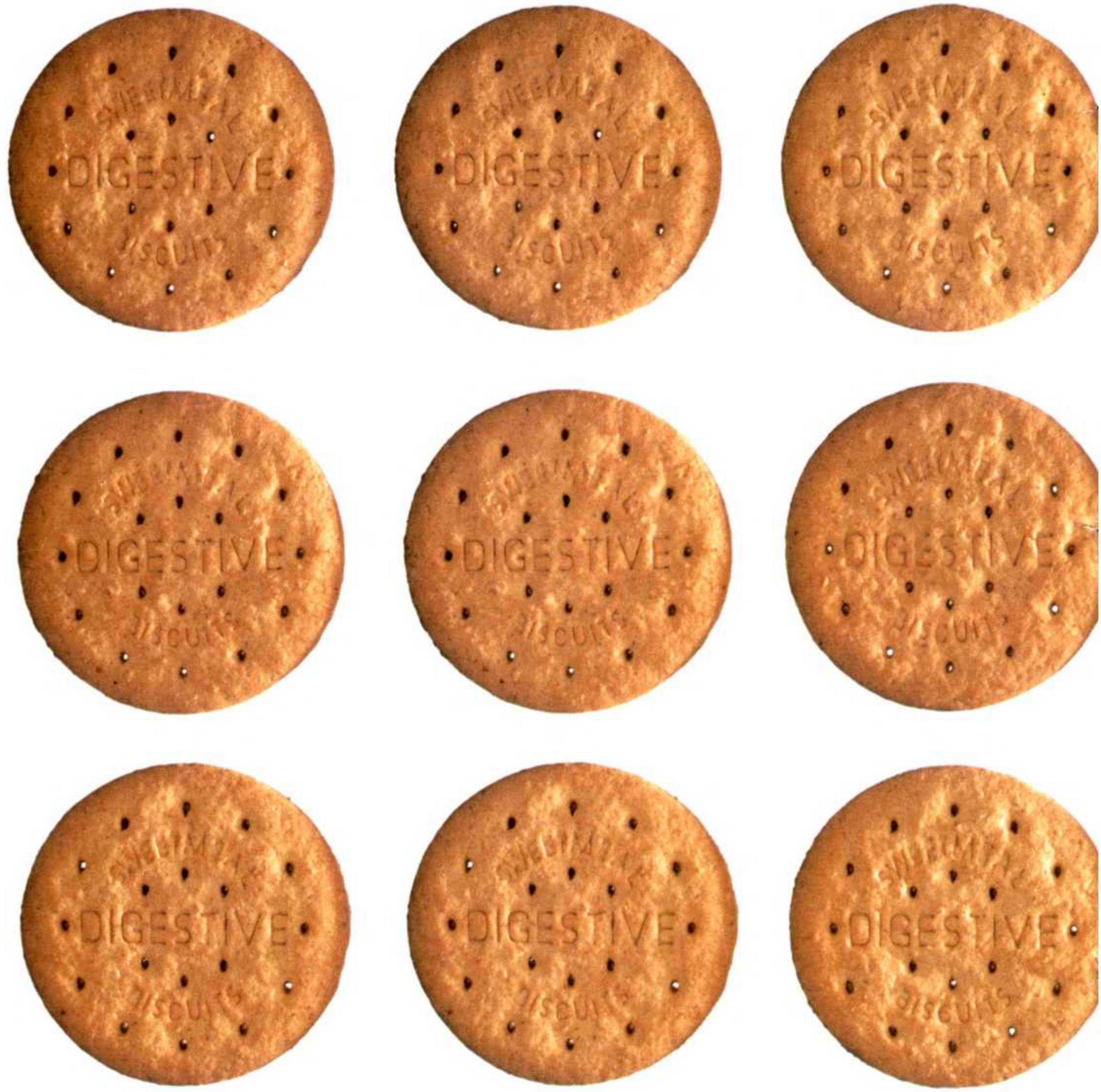

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Intraoperative lymphatic mapping with sentinel lymph node (SLN) biopsy has become a revolutionary concept in the management of solid malignancies. The SLN is defined as the first node draining a regional lymphatic basin. The use of SLN biopsy was first proposed as an alternative to elective lymph node dissection for the staging of regional lymph nodes in patients with cutaneous melanoma by Morton *et al.*[1] in 1992. Although the concept of lymphatic mapping dates back centuries, the minimally invasive nature of this new technique has caused it to be embraced by modern surgeons as they strive for accurate ways of staging patients with solid tumours.

The biopsy technique has been described in patients with melanoma, and in those with breast, thyroid, colon and oropharyngeal carcinoma[2–5]. While the utility of SLN biopsy in thyroid disease remains to be determined, its use for thyroid neoplasia may have some theoretical advantages. If SLN biopsy can accurately detect those patients with occult nodal disease, a selective approach to metastases (with either nodal dissection and/or ^{131}I ablative therapy) could be individualized for each patient.

Identification of nodal disease by palpation in thyroid malignancy has been demonstrated to be inaccurate. It is known that 30–50 per cent of adult patients with well differentiated thyroid cancer have palpable nodal disease, yet up to 90 per cent of patients have histologically proven lymph node metastases after elective modified radical neck dissection[6,7]. Unfortunately, the clinical significance of occult nodal metastases in those with well differentiated disease remains controversial. While several authors have reviewed their experience retrospectively and conclude that compartment orientated nodal dissection decreases locoregional recurrence and improves survival[8], others doubt the clinical significance of occult nodal disease[9].

Kelemen *et al.*[4] were the first to demonstrate that SLN biopsy with vital blue dye staining is feasible in thyroid neoplasia; their study contained 17 patients in whom 15 SLNs were identified. They reported a false-negative rate of 8 per cent and had four patients in whom the lymphatic channels tracked out of the central compartment. However, central neck dissection and postoperative ^{131}I scans were not carried out in the 12 patients with papillary thyroid cancer and so the true false-negative rate was not calculated. The feasibility and safety of SLN biopsy in nodular thyroid disease has also been studied at the University of Calgary[3]. Forty patients with nodular thyroid neoplasia and clinically negative nodal disease underwent SLN biopsy using vital blue dye. Almost 30 per cent of patients had lymphatic channels tracking through the central compartment into the anterior mediastium or the lateral compartment; in 13 per cent the SLN skipped the central compartment. This phenomenon of initial nodal spread lying outside the central compartment had been demonstrated previously. Noguchi and Murakami[7] in 1987 found that 7 per cent of nodal metastasis in well differentiated thyroid cancer appeared only in the lateral compartment. If SLN biopsy were to be useful in thyroid disease, the technique would have to extend beyond the central compartment. The preliminary results of lymphatic mapping in thyroid disease indicate that the surgical dogma that the status of the central nodal compartment is predictive of regional nodal disease might not always be true. It is conceivable that SLN biopsy might refine the surgical dogma that all patients with medullary thyroid cancer should have a total thyroidectomy and central lymph node dissection; it may be possible to include only those patients with proven occult nodal involvement.

Ideally, to assess the accuracy of SLN biopsy would require a large study in which a complete nodal dissection is carried out in all patients following the attempted biopsy. In the Calgary study, all 12 patients with papillary thyroid cancer underwent postoperative ^{131}I scanning and seven patients had nodal dissection[3]. From these data, two false-negative SLN biopsies were identified, plus a third patient in whom a parathyroid gland stained blue while the metastatic lymph nodes within the central compartment did not. Although the number of patients in this series is small, it inevitably raises concern about the accuracy of SLN biopsy in thyroid disease.

The sensitivity of SLN biopsy in detecting occult disease with vital blue dye alone needs to be considered. In the Calgary study, a total of nine patients with papillary thyroid cancer had metastatic nodal disease detected on lymph node dissection or postoperative scanning; the SLN biospy detected only six of these nine patients[3]. In a recent update of the original series by Kelemen *et al.*[4], Haigh and Giuliano[10] reported that occult nodal disease was detected in only nine of their 16 patients with papillary disease. Combining these two series, SLN biopsy detected occult disease in only 15 (54 per cent) of 28 patients with papillary thyroid cancer. Since the incidence of occult nodal disease in this condition is reported to be as high as 90 per cent[6–8], one has to question the sensitivity of the biopsy technique in detecting micrometastasis.

Unlike melanoma, for which SLN biopsy has been validated as an accurate method of assessment of occult nodal disease, it appears from the only two relevant series available that thyroid cancer requires further study in this context. If the accuracy of SLN biopsy can be established and the technique refined, the next step will be to demonstrate a survival benefit to patients who undergo biopsy and subsequent treatment. Randomized trials in both breast cancer and melanoma are presently under way to address the survival benefit associated with SLN biopsy. The application of the information gained from such biopsy in thyroid malignancy will, unfortunately, be harder to define. Endocrine surgeons are plagued by the lack of randomized controlled clinical trials in the treatment of thyroid malignancy and the clinical significance of occult nodal disease in well differentiated thyroid cancer remains controversial. For the surgeon who believes that occult nodal disease plays a clinically significant role in the long-term survival of patients with thyroid cancer, the information gained from SLN biopsy may help to select a population of patients who may benefit from more aggressive surgical resection or ^{131}I ablation therapy. However, such a hypothesis must be tested in a randomized controlled trial to demonstrate the true value of SLN biopsy in thyroid disease.

The role of SLN biopsy has yet to be determined in the thyroid setting. Certainly the technique can be performed safely, but its accuracy needs further investigation before it can be recommended in the routine management of thyroid neoplasia. The onus must fall on the endocrine surgeon to define clearly the direct therapeutic relevance of occult nodal disease if SLN biopsy is to become a standard of care in thyroid cancer.

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Clinical dilemma

Varicose veins and pregnancy

A 28-year-old nulliparous woman with asymptomatic primary varicose veins intends to start a family and asks for advice about treatment of the varicosities. She is not concerned about cosmesis, but wonders if she should have surgery before starting her family or if she should wait and have the matter dealt with, if necessary, at a later stage? Mrs Linda de Cossart of Chester, UK, replies.

Classical teaching on the management of varicose veins in relationship to pregnancy has been that, since pregnancy causes new varicose veins, any definitive treatment should be deferred until child bearing is complete. A recent study of midwives and general practitioners confirms this opinion[1]. It is well known that pregnancy is a time when women first notice their varicose veins[2–4] and traditional teaching is based on the idea that pregnancy causes new lower limb superficial venous reflux resulting in the appearance of new varicosities. There is no evidence to support this theory. Indeed new evidence is emerging to suggest that it is frankly untrue[5–7].

Vascular surgeons are increasingly recognizing that it is necessary to operate between pregnancies on symptomatic varicose veins that have given problems during a pregnancy but this message has not permeated through to other health professionals involved in the care of pregnant women. Nevertheless there is still no consensus on how to manage varicose veins in women before they embark on a pregnancy. The following is my own approach, developed after many years of managing varicose veins.

History and examination

First the vascular surgeon must take a complete history and examine the superficial venous system of the affected limb. This includes both clinical examination with the woman standing and assessment of venous reflux in the long and short saphenous systems with a hand-held Doppler instrument. Sometimes colour duplex examination will also be needed. It is assumed for the rest of this discussion that the woman has uncomplicated long saphenous vein (LSV) reflux although the following comments would also apply to short saphenous vein reflux. The discussion is not about single varicosities or venous flares.

Reflux with minimal distension of the veins must be considered differently from reflux causing bulging varicosities. The latter are more likely to cause problems than the former in the short term. That the woman in this Dilemma is unconcerned about the cosmetic effect of her veins is unusual. One generally finds that young women with visible varicose veins and demonstrable LSV reflux do not like the appearance of their legs. Perhaps this woman feels that she should not make a fuss about a 'cosmetic' rather than an 'abnormal' condition. Still, varicose veins are abnormal and interfere with quality of life[8]. The surgeon should explore this further with this woman.

Communication

The condition and its risks should be explained and the following points should be discussed.

1 Varicose veins are common, affecting up to 15 per cent of the community[9].

2 Varicose veins are caused by an abnormality of the valves in the superficial veins that results in abnormal flow (reflux); the condition is probably congenital.

3 The valvular abnormality cannot be cured.

4 Reflux may result in dilated and unsightly veins that may ache, be tender and hot, may cause swelling of the ankle, and may lead to phlebitis.

5 In a few, lifelong reflux may result in chronic venous insufficiency and its attendant features (lipodermatosclerosis, eczema, pigmentation and gaiter area ulceration).

6 Reflux and venous dilatation increase with age and so varicosities are likely to become more obvious with time.

7 Venous dilatation increases in pregnancy but not all women suffer to the same extent. Support hosiery during pregnancy may be helpful[6,7].

8 No data are available to quantify the risk of pregnancy with varicose veins. This author feels that the most risky complication is phlebitis as there is mounting evidence that extension of the superficial clot into the deep system is more common than previously recognized[10].

9 Treatment may be either non-surgical using support hosiery (which will control the symptoms but not cure the problem) or surgical (a standard operation to remove the abnormal veins). Surgery is usually performed under general anaesthetic but is not normally undertaken during pregnancy.

10 The risk of operation to an otherwise fit woman is very low but mention should be made of wound infection, nerve injury and the remote possibility of deep vein thrombosis. Any operation is associated with discomfort and bruising is virtually inevitable. The woman will need to restrict her normal activities for up to 3 weeks. A good result may be

anticipated in 85 per cent of cases but a few people will develop recurrent varicose veins, the aetiology of which may be aggressive neovascularization associated with the healing process. This is not predictable.

11 Written information should be available for the woman about the facts discussed.

Decision

After examination and an appropriate discussion, the patient and her surgeon should decide on a plan of action. This is likely to be one of the following.

1 The woman may require some time to think about this information and she should be given the opportunity to reflect on it. This will require a further outpatient visit at a later date.

2 The woman may decide not to undergo surgery and to proceed with her first pregnancy. She should be allowed easy access to the vascular surgeon during the pregnancy (should venous complications occur) and thereafter.

3 The woman may wish to undergo early operation and the surgeon must then endeavour to perform the surgery before she becomes pregnant.

Opinion

The author has advised patients on this topic on many occasions. While consultation about phlebitis during or between pregnancies is commoner, there is an increasing tendency for women to seek advice before starting a pregnancy. It seems to me that the biggest influence must be the state of the veins at presentation and this would significantly influence my discussion with the patient. Minimal dilatation would lead me to advise a more conservative approach, but being guided by the woman's choice. Already well dilated veins would lead me to a more interventionist path. The patient's understanding of the pros and cons of the decision process is essential, and it must be remembered that there is no current evidence to lead one to insist on surgery.

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Review

Laparoscopy in the staging of pancreatic cancer

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Background: Over the past decade, laparoscopy has emerged as a popular method of detecting extrapancreatic metastatic disease in patients presumed to have localized pancreatic cancer.
Methods and results: The English language literature on laparoscopic staging of pancreatic cancer was reviewed. Interpretation of this literature on staging laparoscopy is difficult because (1) there has been inconsistent use of high-quality computed tomography (CT) in prospective studies, (2) many studies have included patients with locally advanced disease, and (3) the $R_0/R_1/R_2$ resection rates among patients staged by laparoscopy have not been reported, making it impossible to correlate laparoscopic findings with the R_0 resection rate. Laparoscopy may prevent unnecessary laparotomy in a proportion of CT-staged patients presumed to have resectable pancreatic cancer. However, routine laparoscopy is performed on patients judged to have resectable disease by high-quality CT, this fraction of patients is between 4 and 13 per cent.
Conclusion: When state-of-the-art CT is available, the routine use of staging laparoscopy may not be easily justified from the data in the recent literature. Selective use of laparoscopy may be more appropriate and will probably be a more cost-effective staging approach. Criteria are presented for the selective use of laparoscopy in the staging of patients with localized pancreatic cancer.

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Introduction

Following initial work by Cuschieri and colleagues[1,2], laparoscopy has emerged as a popular method of detecting extrapancreatic metastatic disease in patients presumed to have localized pancreatic cancer. It allows direct inspection of the peritoneal surface and liver to detect occult metastatic disease not seen on preoperative imaging studies. In addition, some investigators believe that this technique can be used to assess local tumour resectability[3]. Favourable preliminary experience with laparoscopy in the identification of subclinical metastatic disease led to a policy of routine laparoscopy in the staging of patients with pancreatic adenocarcinoma in some centres[3,4]. However, this practice is not universal. Indeed, some researchers have reported relatively low rates of occult findings at laparoscopy, leading to the selective use of this staging modality[5–9].

The English language literature on laparoscopy in the staging of pancreatic cancer was reviewed by searching Medline over a 20-year period from January 1980 to December 1999. MeSH (medical subject headings) terms used included pancreatic neoplasms, gastrointestinal neoplasms, laparoscopy, neoplasm staging, endosonography and ultrasonography. A summary was produced with emphasis on laparoscopy for the detection of computed tomography (CT)-occult subclinical metastatic (M_1) disease and/or local tumour extension to the mesenteric vessels. The emerging data on laparoscopic ultrasonography and the role of laparoscopy in patients with locally advanced pancreatic cancer were also reviewed. Finally, the limitations of the existing literature in the context of the quality of prelaparoscopy CT have been addressed and the endpoints used for assessment of the usefulness of laparoscopy have been considered.

Pretreatment staging of pancreatic adenocarcinoma

Staging laparoscopy has been used both as a separate staging study (i.e. requiring a separate general anaesthesia induction from that used for subsequent therapeutic laparotomy)[9,10] and as part of a combined approach in which initial laparoscopy immediately precedes possible laparotomy[3,4,11]. Irrespective of the timing of laparoscopy relative to laparotomy, the yield of laparoscopy is largely a function of where in the staging algorithm the technique is used (*Fig. 1*). If diagnostic laparoscopy is performed early in the evaluation of patients with suspected pancreatic or periampullary cancer (before high-quality contrast-enhanced helical CT), it produces a higher overall yield of positive findings as most patients with pancreatic cancer have locally advanced or metastatic disease at the time of diagnosis. Conversely, the yield of laparoscopy in the detection of M_1 disease is lower if it is applied after high-quality CT. The initial CT scans obtained in patients with suspected pancreatic cancer are often suboptimal, without proper contrast enhancement or tailoring to the anatomical region of interest. High-quality CT images will detect metastatic disease not seen on poor-quality studies, and patients with resectable primary tumours appear to have a lower incidence of occult extrapancreatic metastases than those with locally advanced disease[9]. Thus, the actual yield of laparoscopy cannot be assessed from studies that have not included state-of-the-art prelaparoscopy CT.

Improvements in CT technology have occurred simultaneously with the emergence of laparoscopy as a staging tool in pancreatic cancer. This co-emergence of technology has confounded the interpretation of the literature on the usefulness of laparoscopy. The improvements in CT technology include the development of dynamic scanning, in which contrast material is administered intravenously by automatic injector, and helical CT scanning, which permits the entire pancreas to be imaged during the pancreatic parenchymal phase of intravenous contrast enhancement, when maximal enhancement of the normal pancreatic parenchyma occurs. This approach maximizes the differences in density between the enhancing normal pancreatic parenchyma, the relatively hypodense tumour mass and important adjacent vascular structures (*Figs 2–4*).

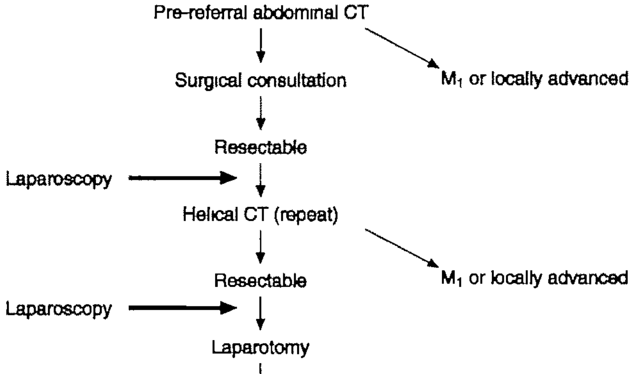

Fig. 1 Hypothetical staging algorithm outlining the different points at which laparoscopy may be employed. The yield of laparoscopy is a function of the point in the staging algorithm at which laparoscopy is applied. Repeat high-quality computed tomography (CT) will identify a subpopulation of patients with metastatic (M_1) or locally advanced disease and so reduce the maximum yield of M_1/locally advanced findings at subsequent laparoscopy

The absence of widespread use of properly performed helical CT, combined with the lack of clear, objective radiographic criteria to determine resectability, has led to variability in published resectability rates (*Table 1*)[3,5,8,9,11–14]. However, when high-quality CT is performed during the bolus phase of contrast enhancement, the accuracy of radiographic prediction of resectability ranges from 75 to 89 per cent[5,7,8,11,12]. Specific, objective, anatomy-based CT criteria have been developed to define potentially resectable pancreatic cancer. These criteria include: (1) the absence of extrapancreatic disease, (2) a patent superior mesenteric vein (SMV)–portal vein confluence (assumes the technical ability for resection and reconstruction in cases with isolated vein involvement), and (3) no direct tumour extension to the coeliac axis or superior mesenteric artery (SMA)[15]. Using these criteria, patients without CT-defined metastatic disease can then be staged as having localized potentially resectable disease, or having locally advanced (radiographically unresectable) disease[15,16]. Prospective use of these objective radiographic criteria has resulted in a radiographic resectability rate (number resected as a proportion of number believed to be resectable by CT) of 80 per cent at this institution[5]. This experience is similar to that reported by other investigators using high-quality CT[5,7,8,11,12,14].

The accuracy of high-quality CT in predicting resectability has important implications with respect to the usefulness of laparoscopy. Since high-quality CT staging can accurately predict resectability in approximately 80 per cent of patients, the maximum positive yield of *routine* laparoscopy as part of a staging algorithm for patients with potentially resectable disease that employs high-quality CT would be 20 per cent. In fact, this figure is an overestimate since it assumes that laparoscopy would be effective in the identification of *all* patients with unresectable tumours because of occult M_1 disease or local tumour extension involving the mesenteric vessels or coeliac axis (it assumes a false-negative rate of zero). This assumption is not supported by recent experience which suggests a false-negative rate of between 3 and 9 per cent for the laparoscopic detection of CT-occult intraperitoneal M_1 disease[3,9,17]. False-negative rates as high as 38 per cent have been observed for the laparoscopic detection of either intraperitoneal M_1 disease or local tumour extension to the mesenteric vessels[17].

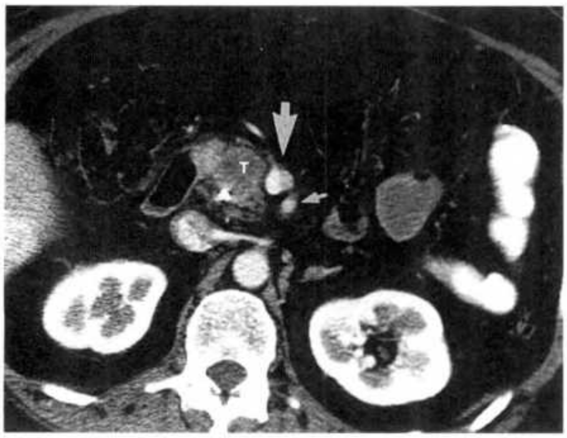

Fig. 2 Contrast-enhanced computed tomogram demonstrating a resectable adenocarcinoma of the pancreatic head. Note the normal tissue plane between the low-density tumour (T) and both the superior mesenteric artery (small arrow) and the superior mesenteric vein (large arrow). The latter is imaged at the confluence of the gastrocolic trunk, which is shown entering the superior mesenteric vein at the 11.30 clock face position

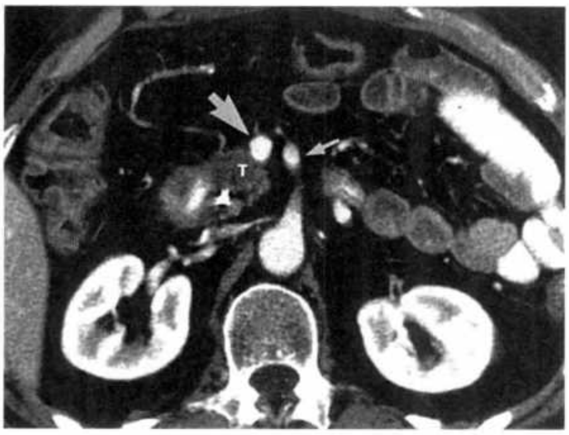

Fig. 3 Contrast-enhanced computed tomogram demonstrating a resectable adenocarcinoma of the pancreatic head, with loss of the normal fat plane between the tumour (T) and the superior mesenteric vein (large arrow). Pancreaticoduodenectomy in such a case commonly requires segmental resection of the superior mesenteric vein. Note the normal tissue plane between the tumour and the superior mesenteric artery (small arrow)

Laparoscopy for detection of computed tomography-occult M_1 disease

It is clear that laparoscopy can prevent unnecessary laparotomy in patients who have CT-occult M_1 disease manifested by laparoscopically detectable peritoneal or surface liver metastases. This is demonstrated by the experience at the Massachusetts General Hospital over the past two decades[9,10]. Investigators from that institution first reported the results of laparoscopic staging in 114 patients with cancer of the pancreatic head ($n = 89$) or body/tail ($n = 25$) and no evidence of distant metastatic disease on CT. Metastases were identified at laparoscopy in 27 patients (24 per cent); occult intra-abdominal metastases were found more often in association with tumours of the body and tail of the pancreas than with tumours of the pancreatic head (44 *versus* 18 per cent; $P < 0.05$). Treatment of the remaining 87 patients was based on the results of postlaparoscopy, preoperative visceral angiography. Of these 87 patients, 42 (48 per cent) had angiographic findings consistent with vascular involvement and were offered non-surgical therapy. The remaining 40 patients (five patients refused surgery or were lost to follow-up) had negative angiographic findings and underwent surgical exploration; 30 of them successfully underwent pancreaticoduodenectomy. This experience clearly emphasizes the usefulness of laparoscopy in detecting occult metastatic disease. It should be noted that the relatively high incidence of CT-occult metastases in this series (24 per cent) is probably related to the substantial number of patients with locally advanced disease who were included in this study; occult metastatic disease is more common in patients with locally advanced (radiographically unresectable) primary tumours than in patients with presumably localized, potentially resectable disease[9]. If high-quality CT had excluded patients with locally advanced disease before laparoscopy, the incidence

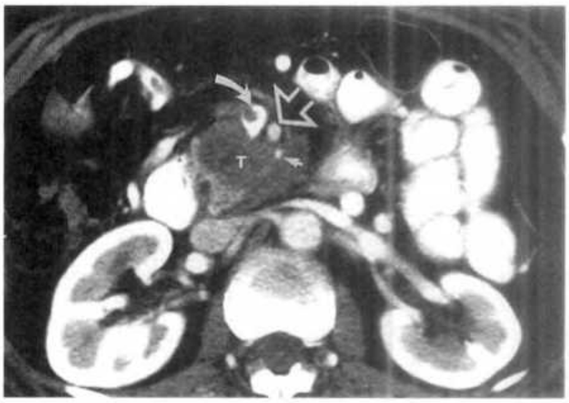

Fig. 4 Contrast-enhanced computed tomogram demonstrating a locally advanced (radiographically unresectable) adenocarcinoma of the pancreatic head. Note the loss of the normal tissue plane between the tumour (T) and the superior mesenteric vein (curved arrow) and artery (large open arrow). There is complete encasement of the posterior aspect of the superior mesenteric artery, and the inferior pancreaticoduodenal artery (small arrow) is seen posterior to the superior mesenteric artery. There is intravascular thrombus identified within the lumen of the superior mesenteric vein

Table 1 Overall and R_0 resection rates in patients considered to have resectable pancreatic cancer based on computed tomography

Reference	Year	Group	Primary site(s)	Helical CT	No. considered resectable	No. resected	Resection rate (%) Overall	Resection rate (%) R_0	Comment
Conlon *et al.*[3]	1996	MSKCC	P (h, b, t), A, D, BD, other	Some	115	61	53	NR	Included 9 patients with non-adenocarcinoma histology, 3 with benign disease and 12 with non-pancreatic periampullary malignancies
Rumstadt *et al.*[12]	1997	Mannheim	P (h, b, t), A	All	194	172	89	NR	Laparoscopy estimated to be potentially beneficial in only 7%
Holzman *et al.*[11]	1997	Duke	P (h, b, t), A, other	All	23	18	78	NR	Overall CT staging accuracy estimated to be 87%; included 9 patients with non-adenocarcinoma histology, 3 with benign disease
Spitz *et al.*[5]	1997	MDACC	P (h), A, BD	All	118	94	80	83*	Included 17 patients with non-pancreatic periampullary malignancies
Friess *et al.*[8]	1998	Bern	P (h, b, t), A, D, BD, other	All	159	119	75	NR	Laparoscopy estimated to be potentially beneficial in 10%; included 55 patients with non-pancreatic periampullary malignancies and 6 with non-adenocarcinoma histology
Steinberg *et al.*[13]	1998	UCLA	P (h)	All	32	24	75	NR	Laparoscopy estimated to be potentially beneficial in 13%
Jimenez *et al.*[9]	2000	MGH	P (h, b, t)	All	42	23	55	NR	Excluded patients with T_1 tumours
Saldinger *et al.*[14]	2000	BI	P (h), A, BD	All	68	52	76	78†	Included 16 patients with non-pancreatic periampullary malignancies

*Fifty (83 per cent) of the 60 patients with pancreatic adenocarcinoma treated with surgery and preoperative or postoperative chemoradiotherapy underwent R_0 resection; †28 (78 per cent) of the 36 patients with pancreatic adenocarcinoma underwent R_0 resection. CT, computed tomography; MSKCC, Memorial Sloan-Kettering Cancer Center; MDACC, The University of Texas M. D. Anderson Cancer Center; UCLA, University of California, Los Angeles; MGH, Massachusetts General Hospital; BI, Beth Israel Hospital, Boston; P, pancreas; h, pancreatic head; b, pancreatic body; t, pancreatic tail; A, ampulla; D, duodenum; BD, bile duct; NR, not reported

of occult M_1 disease found laparoscopically would have been considerably less than the reported 24 per cent.

A more recent report from the same investigators described 125 patients with non-metastatic (localized and locally advanced) pancreatic cancer, as assessed by CT, who underwent staging laparoscopy between 1994 and 1998[9]. Again, the authors did not attempt to separate patients with localized, resectable disease from those with locally advanced disease before laparoscopy because of a belief that patients with locally advanced disease, who could be demonstrated by laparoscopy to have M_1 disease, should be considered for palliative chemotherapy rather than locoregional chemoradiation. Of the 125 patients with presumed M_0 disease (following helical CT), 39 patients (31 per cent) were found to have extrapancreatic disease by laparoscopy (30 patients; 24 per cent) or by cytological assessment of laparoscopically obtained peritoneal fluid (nine patients; 7 per cent). Of the remaining 86 patients, 55 had locally advanced disease (by prelaparoscopy helical CT) and 31 were believed to have resectable pancreatic cancer; 23 of these 31 patients underwent resection. The distribution of patients undergoing resections classified as R_0 (grossly and microscopically negative surgical margins), R_1 (grossly negative but microscopically positive margins) and R_2 (grossly and microscopically positive margins) was not reported, and so it is difficult to evaluate the usefulness of laparoscopy in maximizing the proportion of patients undergoing potentially curative (R_0) resection. The relatively high yield of positive findings at laparoscopy was again due to the inclusion of 83 patients (66 per cent) with locally advanced disease who were clearly not candidates for resection based on CT findings alone, and the inclusion of patients with adenocarcinoma of the body and tail of the gland (47 patients; 38 per cent) who are at greater risk of occult M_1 disease[10,12,18], presumably because of their generally later disease stage at presentation.

Observations from Memorial Sloan-Kettering Cancer Center further demonstrate the usefulness of laparoscopy in the identification of occult M_1 disease. Conlon and colleagues[3] evaluated 115 patients with suspected or proven peripancreatic tumours (103 had pancreatic adenocarcinoma) believed to be resectable based on CT. All patients underwent a detailed laparoscopic examination, including inspection of the lesser sac and selective biopsy of coeliac, portal and/or perigastric lymph nodes. Laparoscopic findings precluding resection were found in 41 (38 per cent) of 108 evaluable patients. The reasons for non-resectability were CT-occult liver metastases in 20 patients, extrapancreatic disease (defined as CT-occult but laparoscopically apparent involvement of the transverse mesocolon) in 16, suspected or proven lymph node metastases in eight and vascular invasion by the primary tumour in 16. Sixty-one (91 per cent) of the remaining 67 patients underwent pancreatic resection. The $R_0/R_1/R_2$ resection rates were not reported for those who had laparoscopic staging and subsequent resection; this makes it difficult to determine the added benefit of laparoscopy in maximizing the fraction of patients who underwent potentially curative (R_0) resection. Overall, only 53 per cent of the initial 115 patients believed to have resectable tumours by preoperative CT underwent resection. Although the authors did not describe the specific CT technique(s) employed for prelaparoscopic staging, this relatively low radiographic resectability rate (in comparison to that achievable with high-quality CT[5,8,9,11–13]) suggests that optimal preoperative CT may not have been used for all patients before laparoscopy.

Recent studies have evaluated the potential yield of laparoscopy in the detection of occult M_1 disease following high-quality CT[5,7,8,11,12,14]. The results of these reports are summarized in *Table 2*. CT-occult M_1 disease was found in only 4–13 per cent of patients with tumours considered resectable following high-quality CT. Therefore, assuming that laparoscopy is 100 per cent accurate in identifying all patients with occult M_1 disease, the maximum percentage of patients who would be spared laparotomy on the basis of laparoscopic detection of M_1 disease using a staging algorithm incorporating routine laparoscopy ranges from 4 to 13 per cent.

Rumstadt and colleagues[12] from the University of Heidelberg illustrate best the maximum potential yield of laparoscopy after high-quality CT. In their report, 398 patients with pancreatic or periampullary cancer were evaluated by high-quality CT, endoscopic retrograde cholangiopancreatography and angiography. Based on this imaging evaluation, 194 patients' tumours were considered 'inoperability unlikely' (i.e. high probability of resection based on the absence of 'infiltration into adjacent tissue or distant metastases'). Of these 194 patients, 172 (89 per cent) underwent pancreaticoduodenectomy. Nine (5 per cent) of the 194 patients believed to have resectable tumours based on high-quality preoperative imaging were found to have occult metastases at laparotomy. The authors concluded that only this small group of patients would have benefited from laparoscopy.

The decrease in incidence of occult M_1 disease at laparotomy that has followed implementation of superior CT technology has been well characterized by several groups[10,18]. The effect of the co-emergence of improved CT technology and laparoscopy has been to confound interpretation of the evolving data evaluating the usefulness of laparoscopy in the staging of patients with pancreatic cancer.

Laparoscopy for detection of local tumour extension to mesenteric vessels

Laparoscopy is also used to identify CT-occult extension of the primary tumour to critical retroperitoneal vascular structures (SMA, SMV and coeliac axis). In the original report from the Massachusetts General Hospital, 42 (48 per cent) of the 87 patients with radiographically resectable disease by CT and negative laparoscopy findings had angiographic findings precluding resection[10]. These initial observations and subsequent additional reports[11,17,19] suggest that standard laparoscopy with intra-abdominal examination of peritoneal surfaces offers limited information about the critical retroperitoneal tumour–vessel relationships. In an effort to address some of the limitations of standard laparoscopy, a more thorough laparoscopic staging technique has been developed and termed 'extended laparoscopy'[3].

Conlon and colleagues[3] at the Memorial Sloan-Kettering Cancer Center performed multiport extended

Table 2 Incidence of occult peritoneal or liver M_1 disease detected at laparotomy after helical computed tomography

Reference	Year	Group	No. considered resectable	Resection rate (%)	No. with M_1 disease	Maximum % spared laparotomy*
Steinberg *et al.*[13]	1998	UCLA	32	75	4	13
Friess *et al.*[8]	1998	Bern	159	75	16	10
Rumstadt *et al.*[12]	1997	Mannheim	194	89	9	5
Holzman *et al.*[11]	1997	Duke	23	78	1	4
Spitz *et al.*[5]	1997	MDACC	118	80	18	15
Saldinger *et al.*[14]	2000	BI	68	76	3	4

*Maximum percentage of patients who would have been spared laparotomy if laparoscopy had been performed with false-negative rate of zero. UCLA, University of California, Los Angeles; MDACC, The University of Texas M. D. Anderson Cancer Center; BI, Beth Israel Hospital, Boston laparoscopy before planned pancreaticoduodenectomy in their series of 115 patients (103 of whom had pancreatic adenocarcinoma). Their technique is more complex than standard laparoscopy and includes assessment of the primary tumour; examination of the liver and porta hepatis; division of the gastrohepatic omentum; examination of the caudate lobe, vena cava, coeliac axis and lesser sac; identification of the ligament of Treitz; and inspection of the mesocolon, duodenum and jejunum. The goal of this technique is to replicate laparoscopically the assessment of resectability performed at laparotomy. Tumours were considered locally advanced and unresectable if laparoscopic examination revealed (1) extrapancreatic extension of tumour (i.e. to the soft tissues of the transverse mesocolon), (2) coeliac or portal lymph node involvement, (3) tumour invasion or encasement of the coeliac axis or hepatic artery, or (4) tumour encasement of the portal vein, SMV and/or SMA. Although some of these criteria (i.e. nodal involvement and isolated involvement of the SMV or portal vein) are not universally viewed as contraindications to resection, they were used to identify extrapancreatic tumour extension in 16 patients (sole site of disease in three patients), nodal disease in eight patients (sole site of disease in one patient) and vascular invasion (vessel unspecified) in 16 patients (sole site of disease in ten patients). In the 14 patients (12 per cent) in whom laparoscopic identification of extrapancreatic tumour extension, nodal disease or vascular invasion was the only criterion for determining unresectability, extended laparoscopy obviated laparotomy.

Other investigators have questioned the value of laparoscopy in the detection of mesenteric or retroperitoneal vascular involvement. Their concern is based on the obvious limitation of an *intraperitoneal* technique relying on surface inspection for detection of *retroperitoneal* and/or *mesenteric* tumour spread[12]. There is doubt as to whether laparoscopic inspection of a portion of the portal vein through the foramen of Winslow or laparoscopic examination of the gastrohepatic omentum, lesser sac and coeliac axis can accurately replace operative dissection and the exposure it affords of these critical tumour–vessel relationships[12,17,20]. Jimenez and colleagues[9] have also noted that laparoscopic replication of the open exploration for pancreatic cancer, including dissection of the lesser sac and SMV, is time consuming, potentially dangerous and beyond the skills of the non-expert.

The interpretation of studies examining staging techniques and their ability to predict resectability of pancreatic or periampullary neoplasms is difficult because of a lack of clear anatomical description of the basis of unresectability (*Table 3*). Moreover, it is possible that the threshold for declaring local tumour unresectability on the basis of isolated involvement of the SMV or portal vein may be a function of the surgeon's experience with vascular resection and reconstruction during pancreaticoduodenectomy. Several groups have reported their experience with resection and reconstruction of the SMV and/or portal vein during pancreaticoduodenectomy[15,21–26]. Interestingly, in centres where high-quality imaging is combined with significant experience in vascular resection and reconstruction, it is rare to find patients whose tumours are deemed unresectable at laparotomy because of occult locoregional tumour extension not appreciated on preoperative imaging. For example, Spitz *et al.*[5] recently reported on 118 patients deemed to have resectable tumours following high-quality preoperative CT with objective radiographic criteria for resectability. Only two patients (2 per cent) were found at surgery to have unresectable tumours because of local tumour extension not identifiable by CT; 25 patients (21 per cent) underwent vascular resection and reconstruction because of local tumour adherence to or involvement of major blood vessels. The ability of laparoscopic intraperitoneal inspection further to discriminate critical details of

Table 3 Frequency of occult vascular involvement precluding resection and vascular resection rates after staging by helical computed tomography

Reference	Year	Group	No. considered resectable	No. resected	Resection rate (%)	Locally advanced precluding resection	Vascular resection
Steinberg *et al.*[13]	1998	UCLA	32	24	75	4 (13)*	NR
Friess *et al.*[8]	1998	Bern	159	119	75	17 (11)†	NR
Rumstadt *et al.*[12]	1997	Mannheim	194	172	89	20 (10)‡	NR
Holzman *et al.*[11]	1997	Duke	23	18	78	1 (4)§	NR
Spitz *et al.*[5]	1997	MDACC	118	94	80	2 (2)	25 (21)
Saldinger *et al.*[14]	2000	BI	68	52	76	13 (19)	1 (1)

Values in parentheses are percentages. *'Locally extensive tumour considered unresectable', vessels not defined; †'Tumour infiltration into retropancreatic vessels', vessels not defined; ‡'Locally unresectable', vessels not defined; §'Vascular invasion', vessels not defined. UCLA, University of California, Los Angeles; MDACC, The University of Texas M. D. Anderson Cancer Center; BI, Beth Israel Hospital, Boston; NR, not reported retroperitoneal tumour extension in the small number of patients with CT-occult extrapancreatic extension is questionable. So, when high-quality CT is combined with a specific, objective, anatomy-based definition of local tumour resectability and the technical ability to perform resection and reconstruction for isolated involvement of the SMV or portal vein, the discovery at laparotomy of unsuspected local tumour extension precluding resection is likely to be rare.

Laparoscopy combined with laparoscopic ultrasonography

The limitations of laparoscopy in the staging of pancreatic cancer include: (1) the technique allows only two-dimensional inspection of the surface of the liver and peritoneal cavity; (2) it does not allow palpation of the liver or peripancreatic region, limiting the identification of small intraparenchymal hepatic metastases; and (3) by laparoscopic inspection of the peritoneal surfaces, the critical retroperitoneal tumour–vessel relationships cannot be evaluated accurately. For these reasons, laparoscopic ultrasonography has been proposed as a means of overcoming some of the limitations of two-dimensional laparoscopy and of improving the diagnostic yield over that of laparoscopy alone.

The experience of laparoscopy combined with ultrasonography in the staging of pancreatic cancer is summarized in *Table 4*. Comparison of these series to better define the contribution of ultrasonography to standard laparoscopy and to the overall staging process is difficult because of differences in the patient populations studied, and in the type and quality of prelaparoscopy imaging. Some investigators focused on patients with presumed localized, potentially resectable disease[17,28], whereas others used the absence of M_1 disease on CT as a study entry criterion (including patients with both localized and locally advanced disease)[4]. This makes it difficult to evaluate the contribution of laparoscopy plus ultrasonography in the staging of patients with localized (resectable) disease.

Calculated radiographic resectability rates in the studies of laparoscopy plus ultrasonography range from 25 to 62 per cent. Given the resectability rates that can be achieved by high-quality CT alone (as outlined above and summarized in *Table 1*), these lower resectability rates suggest that relatively poor-quality CT was performed before laparoscopy with ultrasonography. Indeed, in one study, prelaparoscopy CT was not performed in all patients[17]. Studies of laparoscopic ultrasonography are therefore difficult to evaluate because of their failure to use high-quality helical CT staging and objective CT criteria to define the term 'resectable' pancreatic cancer.

Notwithstanding the limitations of the existing literature, several preliminary observations are noteworthy. First, there is some evidence that laparoscopic ultrasonography may increase the yield of laparoscopy, particularly in the detection of subsurface hepatic metastases. John and colleagues[17] evaluated 40 patients with potentially resectable pancreatic cancer as determined by CT. A variety of CT techniques and equipment was employed by the referring institutions participating in the study; CT was repeated only if the original scans were considered to be of inadequate quality. Of the 40 patients who underwent laparoscopy, 14 had identification of unsuspected M_1 disease in the liver, on the peritoneal surface and/or in hilar lymph nodes. Laparoscopy failed to detect M_1 disease (false negative) in three patients (8 per cent). Laparoscopic ultrasonography was performed in 38 patients and was able successfully to visualize the primary tumour in 31. In 20 patients, information relevant to the assessment of tumour stage not apparent from CT or laparoscopy was derived from the ultrasonographic examination. The additional data provided by ultrasonography affected decision making about resectability in ten patients. These findings suggest that ultrasonography may add to the overall sensitivity of

Table 4 Laparoscopy with laparoscopic ultrasonography in the staging of pancreatic cancer

Reference	Year	Group	Prelaparoscopy helical CT	Clinical stage	No. operated	M_1 (%) (Lap + LUS)	No. false negative (Lap + LUS)	Resection rate (%)*
Bemelman *et al.*[27]	1995	Amsterdam	No CT	US T_1	73	16	5	40
John *et al.*[17]	1995	Edinburgh	Some	Localized	40	14	3	25
Callery *et al.*[28]	1997	WU	Most	Localized	32	NR	NR	NR
Minnard *et al.*[4]	1998	MSKCC	All	Localized + locally advanced	90	26	1	62†

*Number resected/number believed resectable after preoperative radiographic staging; †40 resected of 65 with resectable tumours on preoperative imaging. CT, computed tomography; Lap + LUS, laparoscopy and laparoscopic ultrasonography; US T_1, transabdominal ultrasonographic stage T_1; WU, Washington University; MSKCC, Memorial Sloan–Kettering Cancer Center; NR, not reported for pancreatic cancer subset conventional laparoscopy. However, examination of the radiographic resectability rate in this report (ten of 40 patients) again suggests that the quality of prelaparoscopic CT was inferior to that of currently available technology. In fact, this resectability rate is similar to that achieved in the 1980s with conventional (non-helical) CT alone without laparoscopy[29,30], suggesting that a substantial proportion of patients included in the series by John *et al.*[17] had locally advanced pancreatic cancer. Indeed, six of the ten patients in whom ultrasonography affected decision making regarding resectability had tumours greater than 5 cm in diameter, eight had sonographically detected invasion of the SMV or portal vein which in some cases was severe enough to produce angiographic abnormalities, and six had enlarged regional lymph nodes (not biopsied).

Minnard and colleagues[4] have reported their experience with the combination of laparoscopy and laparoscopic ultrasonography in a series of 90 patients. The study population included patients believed to have localized, potentially resectable disease ($n = 65$), patients with locally advanced, radiographically unresectable disease ($n = 17$), and patients with preoperative dynamic CT findings believed to be equivocal with regard to resectability ($n = 8$). The authors performed multiport extended laparoscopy, as originally described by Conlon *et al.*[3], combined with ultrasonography. After multiport extended laparoscopy, 36 patients (40 per cent) were found to have resectable disease, 41 patients (46 per cent) were found to have unresectable disease and 13 patients (14 per cent) had equivocal laparoscopic findings with respect to resectability. Ultrasonography confirmed the findings of extended laparoscopy for all 36 patients judged to have resectable disease (all of whom underwent tumour resection; $R_0/R_1/R_2$ resection rates not reported) and for all 41 patients believed to have unresectable disease (number confirmed at laparotomy not reported). It appeared useful in further assessing resectability in the 13 patients who had equivocal findings at laparoscopy, 12 of whom were suspected to have vascular involvement and one of whom was suspected to have nodal disease. Eight of these 13 patients were found to have unresectable disease by ultrasonography (four of eight confirmed by laparotomy). The majority of these patients had ultrasonographically detected extrapancreatic tumour extension to the coeliac axis or its visceral branches, or to the SMA; three patients declared to have unresectable disease had involvement of the portal vein without arterial extension and so may have been considered for resection with venous reconstruction by other investigators. One of the 13 patients categorized as having equivocal laparoscopy findings had false-negative ultrasonographic findings. Minnard and colleagues did not describe the CT findings in the 13 patients in whom ultrasonography was believed to be helpful, i.e. whether they were originally categorized (by preoperative CT) as resectable, unresectable or equivocal. The inclusion of patients with locally advanced disease and the absence of ultrasonographic results stratified by preoperative CT findings preclude definitive conclusions regarding the contribution of laparoscopic ultrasonography to the staging of patients with localized, potentially resectable disease.

Given the small number of patients studied and the limitations of the existing literature on laparoscopic ultrasonography (*Table 4*), further experience with this technique in the staging of pancreatic cancer is clearly needed before a conclusion about its role can be reached. The aggregate data suggest that it may be of use in patients who are found to have marginally resectable lesions by prelaparoscopy CT or in those who have equivocal findings with respect to resectability at the time of laparoscopy. However, this patient population is relatively small (14 per cent in the Memorial Sloan–Kettering experience), and the technique adds further costs to the laparoscopy and requires significant expertise in interpretation of laparoscopically derived ultrasonographic images. At this time, laparoscopic ultrasonography cannot be considered as a recommended component of the laparoscopic staging of pancreatic cancer.

Laparoscopy for selection of palliative treatments

Laparoscopy for locally advanced disease

The investigators from the Massachusetts General Hospital have included patients with locally advanced (radiographically unresectable) pancreatic cancer in their studies of staging laparoscopy[9,31]. The rationale for this is the high incidence of CT-occult liver and peritoneal metastases (34 per cent) in patients with locally advanced disease and the local practice of treating such patients with systemic therapy rather than locoregional chemoradiation[9]. Patients with peritoneal carcinomatosis and/or liver metastases are, therefore, spared the potential toxicities and time commitment required for more intensive locoregional chemoradiation (usually given from Monday to Friday for 5·5 weeks). The available natural history data suggest that patients with CT-occult extrapancreatic metastases (diagnosed at laparoscopy or laparotomy) have a median survival of approximately 6–7 months[16,32,33]. A similar survival duration is noted in those treated with preoperative chemoradiation and found to have extrapancreatic metastases (following chemoradiation) before planned pancreatectomy[6]. This 6–7-month median survival contrasts with the 9–10-month median survival typical of the chemoradiation trials conducted by the Gastrointestinal Tumor Study Group in patients with locally advanced pancreatic cancer[34–37]. When laparoscopic staging is performed in those with CT-defined locally advanced disease, it is done on the assumption that a median survival duration of 6–7 months does not justify the use of locoregional chemoradiation but that a median survival duration of 9–10 months might justify the added time commitment, potential therapeutic benefit and risk of treatment-related toxicity associated with locoregional chemoradiation. This hypothesis has not been evaluated in controlled trials. Indeed, the optimal treatment approach for patients with locally advanced pancreatic cancer is presently unknown. It is an area where considerably more clinical research with outcome analysis and quality-of-life endpoints is needed.

Laparoscopy for assessment of options for palliation

Luque de León and colleagues[33] from the Mayo Clinic have advocated the use of staging laparoscopy as a means of determining whether patients should undergo surgical or non-surgical palliation of obstructive jaundice. These investigators evaluated the long-term survival of 148 patients with radiographically resectable pancreatic cancer who were found to have unresectable disease at surgery. The median survival duration of patients with CT-occult liver metastases, peritoneal carcinomatosis, distant nodal involvement (defined as clinically evident and histologically confirmed metastatic adenopathy along the common hepatic artery or coeliac axis or at the base of the small bowel mesentery) and local vascular involvement was 6, 7, 11 and 11 months respectively. The difference in survival between patients with liver or peritoneal metastases and those with distant nodal disease or locally advanced disease was statistically significant ($P < 0{\cdot}03$). On the basis of these observations, the authors advocate surgical biliary (and duodenal) bypass for the subset of patients with locally advanced disease, and endoscopic endobiliary stent placement in patients with liver metastasis or peritoneal carcinomatosis owing to their short median survival of 6–7 months. The development of more durable endobiliary stents may alter this recommendation in the future. Luque de León *et al.* also point out that if one adopts a palliative treatment algorithm based on these observations, the main advantage of laparoscopy lies in its ability rapidly to identify patients with CT-occult liver and peritoneal disease. Extended laparoscopy (as defined by Conlon *et al.*[3]), with examination of the lesser sac and attempts to identify tumour–vessel relationships, is not necessary using the Mayo Clinic diagnostic and treatment algorithm because patients with localized pancreatic cancer undergo laparotomy for either resection or palliative biliary bypass.

Endpoints for evaluation of the usefulness of laparoscopy

Interpretation of the literature on laparoscopic staging of pancreatic cancer is confounded by the variable endpoints used to evaluate the usefulness of the procedure. Endpoints employed have included (1) resectability rates (number resected as a proportion of the number believed to be resectable following laparoscopy)[3,4,8,14,17,19], (2) proportion of patients spared non-therapeutic laparotomy[3,7,8,19], (3) number of patients who benefited from laparoscopy (laparotomy avoided) compared with number of patients undergoing unnecessary laparoscopy (laparoscopy plus laparotomy)[8], and (4) cost–benefit comparisons[11]. While all of these criteria are important individually, they should be evaluated in the context of the quality of prelaparoscopy imaging and in the context of the surgical margin status following pancreaticoduodenectomy.

Comparison of resectability rates

Many investigators have defined resectability rate as the number of patients who undergo resection divided by the number of patients who undergo laparotomy (following complete staging including laparoscopy)[3–5,8,14]. While it is clearly desirable to minimize the number of patients who undergo unnecessary laparotomy, it may be equally important to quantify the number of patients who undergo tumour resection relative to the number of patients who undergo an invasive staging procedure requiring general anaesthesia (i.e. laparoscopy or laparotomy), particularly when laparoscopy is performed under a separate general anaesthesia induction. This may be defined alternatively as the 'radiographic resectability rate', i.e. the number of patients who undergo successful resection divided by the number of patients believed to have resectable disease following optimal prelaparoscopy non-invasive staging. Consideration of the radiographic resectability rate is important in evaluating the literature on staging laparoscopy. For example, review of the experience with extended laparoscopy at Memorial Sloan–Kettering revealed a resectability rate (number resected/number undergoing laparotomy) of 91 per cent[3]. However, the radiographic resectability rate in the same series was 53 per cent (61 of 115 patients), considerably lower than the radiographic resectability rates that can be achieved by high-quality CT staging alone (*Table 1*). Investigators should be encouraged both to report specifically radiographic resectability rates and to report separate radiographic resectability rates for pancreatic adenocarcinoma and non-pancreatic periampullary lesions (since these rates are clearly quite different[14]). This allows an indirect evaluation of the quality of prelaparoscopy CT imaging, assessment of the resectability rate for pancreatic adenocarcinoma (as distinct from other periampullary carcinomas) and assessment of whether the study included significant numbers of patients with locally advanced disease. These three factors are important in evaluating the literature on laparoscopic staging of patients with localized pancreatic cancer.

An oncologically significant issue has been obscured by the emphasis on resectability rates. The focus of preoperative imaging of the primary tumour (in addition to identifying extrapancreatic disease) is the definition of the relationship of the low-density tumour to the SMA and coeliac axis. Patients with arterial involvement are considered to have locally advanced, unresectable disease best managed non-operatively. This issue is of critical importance since patients who undergo pancreaticoduodenectomy with a positive retroperitoneal margin of resection at the level of the SMA or coeliac axis have a median survival of approximately 10 months[29,38–44] (*Table 5*); this is no different from that which can be achieved with non-operative combined-modality treatment of more advanced stage disease[34,36,45]. Therefore, the simple recording of whether or not the patient underwent some form of tumour resection should not be used as an endpoint in studies of prelaparotomy staging procedures. Instead, since a major aim of preoperative imaging and laparoscopy is accurate definition of the tumour–vessel relationship, the primary endpoint of prelaparotomy staging studies that include laparoscopy should be the pathological status of the retroperitoneal margin along the SMA (i.e. the $R_0/R_1/R_2$ resection rate). Stated otherwise, the primary goal of prelaparotomy staging should be to maximize the proportion of patients who undergo potentially curative (R_0) resection. This oncologically critical issue has received minimal emphasis in the literature. Indeed, with few exceptions[5,14,15], information on resection margin status is absent from the literature on pretreatment staging and laparoscopy (*Table 1*); incomplete resections may thus be under-reported. The lack of accurate pathological assessment of pancreaticoduodenectomy specimens (i.e. no assessment or an imprecise definition of the retroperitoneal margin) and the lack of subsequent correlation with preresection staging studies are major concerns in the interpretation of the large body of literature on the accuracy of radiographic and/or laparoscopic staging before pancreaticoduodenectomy.

Table 5 Median survival following R_1 or R_2 pancreaticoduodenectomy

Reference	Year	No. of R_1 or R_2 resections	Positive margin	Median survival (months)
Tepper *et al.*[38]	1976	17	R_1/R_2	8
Trede *et al.*[29]	1990	54	R_1/R_2	10
Whittington *et al.*[39]	1991	19	R_2	—*
Willett *et al.*[40]	1993	37	R_1/R_2	11
Nitecki *et al.*[41]	1995	28	R_2	9
Yeo *et al.*[42]	1995	58	R_1/R_2	10
Lillemoe *et al.*[43]	1996	64	R_1/R_2	12
Breslin *et al.*[44]	2000	16	R_1	25†

*Two patients alive at 18 months' follow-up; †following preoperative chemoradiotherapy. R_1, microscopically positive margin; R_2, grossly positive margin

Positive laparoscopy findings *versus* negative laparoscopy findings

A high proportion of patients who undergo laparoscopy following high-quality preoperative imaging will have negative laparoscopic findings, i.e. laparoscopic results do not change the therapeutic plan. Thus, a better index of the usefulness of laparoscopy may be the ratio of patients undergoing laparoscopy that results in a change in the therapeutic plan to patients undergoing unnecessary laparoscopy[8]. Friess and colleagues[8] have employed this type of analysis in evaluating the possible benefit of laparoscopy in 254 patients with pancreatic or peripancreatic malignancies. In their report, the ratio of patients who benefited from laparoscopy (avoided unnecessary laparotomy) to patients who did not benefit from this approach (underwent laparoscopy plus laparotomy) was 1 : 10 for all patients with peripancreatic malignancies and 1 : 7 for patients with pancreatic cancer. In other words, seven unnecessary laparoscopies would have to be performed to avoid one laparotomy. The authors concluded that a ratio of 1 : 7 is too small to recommend routine diagnostic laparoscopy in the preoperative evaluation of patients with pancreatic cancer.

Cost–benefit analysis

Given the recent attention that has been focused on cost containment in medicine and the possible advantages and disadvantages of laparoscopy in this regard, it is remarkable that so few data exist on the economic impact of laparoscopy when employed as part of the staging of patients with pancreatic cancer. Friess and colleagues[8] observed that in a cost–benefit analysis independent of specific hospital charges, routine diagnostic laparoscopy (with its relatively low yield) would need to be seven times cheaper than laparotomy to be justifiable from a cost–benefit standpoint. In their institution, diagnostic laparoscopy costs half as much as diagnostic laparotomy, leading the authors to conclude that from an economic standpoint routine diagnostic laparoscopy could not be recommended.

Cost–benefit analyses of laparoscopy should also take into account the quality of prelaparoscopy imaging. It is not cost effective to use laparoscopy as an alternative to high-quality CT in a patient who is referred with marginal-quality CT scans that demonstrate a 'resectable' pancreatic tumour; CT should be repeated in such patients using a specific scanning protocol tailored for pancreatic tumour imaging. Given the relatively low cost of CT (compared with laparoscopy, which requires general anaesthesia), it will probably be difficult to demonstrate an economic advantage to routine laparoscopy, particularly when performed as a staging procedure under a separate anaesthesia induction before consideration of laparotomy.

Summary and recommendations

Advances in the quality of preoperative imaging have led to accurate radiographic prediction of resectability in 75 per cent or more of patients with pancreatic cancer (*Table 1*). This fact has diminished the usefulness of laparoscopy when employed as a routine prelaparotomy procedure (particularly when performed under a separate general anaesthesia induction) in the staging evaluation of patients with pancreatic cancer. The literature evaluating the use of staging laparoscopy is difficult to interpret because (1) there has been inconsistent use of high-quality CT as an entry criterion for prospective studies, (2) the term 'resectable' has not been defined using objective CT criteria, (3) studies have included patients with non-pancreatic periampullary malignancies and benign disease, (4) many reports have included patients with clear evidence of locally advanced pancreatic adenocarcinoma on staging CT, and (5) there has been no pathological analysis of the retroperitoneal margin of resection in the subset of patients with pancreatic adenocarcinoma, making it impossible to correlate laparoscopic findings with R_0, R_1 and R_2 resection rates.

The aggregate data and clinical experience clearly demonstrate that laparoscopy may prevent unnecessary laparotomy in an occasional patient who is presumed to have resectable pancreatic cancer based on CT findings. However, if laparoscopy is performed on patients judged to have resectable disease by high-quality CT (using objective criteria), the proportion of such patients is in the range of only 4–13 per cent (*Table 2*). The yield of laparoscopy is sufficiently low that its use as a *routine* staging procedure (particularly as a separate staging procedure requiring a separate general anaesthesia induction) cannot be readily justified. Selective use of staging laparoscopy at the time of planned laparotomy for tumour resection may be more appropriate and is likely to be more cost effective.

Further studies are required to define more precisely the subsets of patients with ostensibly localized pancreatic adenocarcinoma in whom laparoscopy should be employed selectively. On the basis of currently available data, patients who may be at higher risk for occult M_1 disease may include those with: (1) larger primary tumours (the precise primary tumour size at which the risk of occult M_1 disease justifies the added time and cost of laparoscopy is currently unknown); (2) lesions in the neck, body or tail of the pancreas; (3) equivocal radiographic findings suggestive of occult M_1 disease, such as low-volume ascites, CT findings indicating possible carcinomatosis, and small hypodense regions in the hepatic parenchyma that suggest hepatic metastases that are not amenable to percutaneous biopsy; and (4) subtle clinical and laboratory findings suggesting more advanced disease (e.g. marked hypoalbuminaemia and/or weight loss, significant increases in CA19-9 level, and relatively severe pain requiring narcotic analgesia). Such patients should be considered for further staging by laparoscopy, either as a separate staging procedure or before planned pancreaticoduodenectomy.

Subsequent studies evaluating the prelaparotomy staging of patients with pancreatic cancer should include high-quality, pancreas-specific CT scanning protocols, assessment of outcomes in terms of cost effectiveness, resection results stratified by histology, and determination of R_0, R_1 and R_2 resection rates as important endpoints for analysis.

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Preoperative (neoadjuvant) chemoradiotherapy in oesophageal cancer

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Background: Oesophageal cancer carries a poor prognosis. The 5-year survival rate following resection ranges from 10 to 35 per cent. Recent evidence suggests that the addition of non-surgical treatments to surgery may improve resection rates, reduce the risk of recurrence and improve survival. This review examines the role of preoperative chemoradiotherapy (CRT) in oesophageal cancer.

Methods: A Medline-based literature review (1980–2000) was performed using the key words 'neoadjuvant or preoperative' and 'chemoradiotherapy or radiochemotherapy'. Additional literature was obtained from original papers and published meeting abstracts.

Results: Forty-six non-randomized and six randomized trials of preoperative CRT were found. Resection rates, pathological complete response (pCR), treatment-related mortality rates and relapse patterns are documented. Improved 5-year survival rates approaching 60 per cent may be achieved following pCR. Three of the six randomized trials show a benefit in either overall survival or disease-free survival compared with surgery alone. Treatment-related toxicity can be significant.

Conclusion: Preoperative CRT may improve survival. Emerging evidence suggests that CRT alone can achieve similar survival rates to surgery alone. New imaging modalities may help to select which patients require surgery. Larger randomized trials of preoperative CRT or chemotherapy are needed to define optimal regimens and produce higher pCR rates with acceptable toxicity.

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Introduction

The worldwide incidence of oesophageal cancer is increasing, particularly adenocarcinoma of the lower oesophagus, gastro-oesophageal junction and gastric cardia[1–3], which is now more common than squamous carcinoma in the UK and USA[4]. A literature review of 83 783 patients treated between 1953 and 1978 showed that the overall 5-year survival rate of patients with oesophageal cancer referred for surgery was 4 per cent[5]. A similar review of 46 692 patients treated between 1980 and 1988 showed a small improvement in overall 5-year survival rate to 10 per cent[6]. However, these data may not be entirely reliable as they are subject to selection and publication bias. In those not suitable for surgery, radical radiotherapy can result in long-term survival. A review of 8489 patients treated by radical radiotherapy between 1954 and 1979 showed an overall 5-year survival rate of 6 per cent[7]. Survival rates following radiotherapy are not directly comparable to those following resection, as patients receiving radiotherapy have tended to have unfavourable risk factors for major surgery and also have not been surgically staged. Three randomized trials comparing radiotherapy with surgery in resectable oesophageal cancer have been attempted. A UK Medical Research Council (MRC) trial in 1991 failed to accrue[8], but two subsequent trials showed a survival benefit in favour of surgery despite recruiting small numbers of patients[9,10].

At diagnosis, surgery is inappropriate in 40–60 per cent of patients owing to irresectable primary disease, distant metastases or poor operative risk[11,12]. Of those undergoing operation, between 55 and 80 per cent will have a 'potentially' curative resection[6,11,12]. The reported postoperative mortality rate in most recent series ranges from 10 to 15 per cent[6] but in specialist units, where large numbers of such operations are performed by a team of experienced surgeons, the value is nearer 5 per cent[13–16]. Age[17], poor performance status[18] and abnormal physiological function (cardiac, respiratory and hepatic)[19,20] are known factors that increase the risk of complications and death following oesophagectomy.

The 5-year survival rate of patients who have undergone curative resection remains disappointingly low, averaging 20 per cent in most series but varying from 10 to 35 per cent[6,12,13,19,21]. Prognostic factors include the degree of oesophageal wall penetration (which correlates with the risk of nodal involvement)[22], the number of lymph nodes involved[23–25] and the site of the primary tumour; lower oesophageal tumours carry a worse prognosis than mid or upper oesophageal tumours[26,27]. Despite improvements in operative surgery, intensive care medicine and supportive care, no significant increase in overall survival has been achieved. Both distant metastases and locoregional recurrence occur equally commonly[13,21,25,28]. Extended lymphadenectomy may improve locoregional control but does not seem to affect overall survival[29]. A plateau in the effectiveness of surgical resection in oesophageal cancer may well have been reached. Further improvement in survival from a single-modality approach seems unlikely[6].

In an attempt to improve the results of surgery, preoperative (neoadjuvant) and postoperative (adjuvant) treatment with chemotherapy, radiotherapy or a combination of both has been used. The potential advantages of a neoadjuvant approach include increasing the rate of curative resection by tumour downstaging and debulking, earlier treatment of micrometastases, and more reliable delivery of non-surgical therapy by avoiding the prolonged recovery period following major surgery. In addition, there are biological hypotheses of decreased risk of tumour seeding at operation and increased radiosensitivity owing to better tumour oxygenation if radiotherapy is given before operation. Finally, there is the unique opportunity to evaluate the effectiveness of the preoperative treatment by histological examination of the resected oesophagus, with pathological complete response (pCR) being defined as the inability of the pathologist to identify viable malignant cells within the specimen. However, the success of any neoadjuvant approach depends entirely on the effectiveness of the treatment regimen used. There is a balance between potential delay of definitive surgery in patients failing to respond to neoadjuvant treatment, potential for increased treatment-related morbidity and mortality rates, and potential for improved locoregional control and survival in those who respond to neoadjuvant treatment. In addition, patients destined for early relapse owing to occult metastatic disease may avoid surgical resection.

Preoperative radiotherapy

Of the five published randomized trials comparing preoperative radiotherapy followed by surgery with surgery alone, none has demonstrated an improvement in resectability rate or overall survival[30–34]. A meta-analysis of these trials using updated individual patient data has also failed to show a statistically significant survival benefit from preoperative radiotherapy[35]. In another trial, 96 patients were randomized to preoperative radiotherapy or preoperative chemotherapy followed by surgery[36]. Again there was no difference in resectability or survival rate between the two arms.

Preoperative chemotherapy

Trials of preoperative cisplatin and 5-fluorouracil (5-FU)-based chemotherapy consistently resulted in clinical response rates of 45–70 per cent[37–41]. Early randomized trials comparing preoperative chemotherapy followed by surgery[34,42], or preoperative and postoperative chemotherapy[43], with surgery alone showed no evidence of an improvement in survival. However, these were small trials that had the power to detect only large differences in outcome. One study[42] was terminated prematurely because of increased postoperative morbidity and mortality rates in the chemotherapy arm. A meta-analysis of published randomized trials comparing chemotherapy with no chemotherapy when combined with surgery or radiotherapy showed no survival benefit from cisplatin-based chemotherapy in oesophageal cancer[44].

More recent larger trials report mixed results. The Intergroup Trial (INT 0113)[45] randomized 467 patients with resectable oesophageal squamous carcinoma and adenocarcinoma to chemotherapy and surgery or to surgery alone. Chemotherapy consisted of three preoperative cycles of cisplatin and 5-FU followed by two postoperative cycles. There was no difference in the rates of resection or postoperative mortality between the two arms. Median (15 *versus* 16 months respectively) and 3-year (23 *versus* 26 per cent respectively) survival were also similar. Toxicity and compliance with this regimen was a major problem, with 54 per cent of patients experiencing grade 3–4 toxicity; only 71 per cent received the complete preoperative course of chemotherapy. The documented clinical response to chemotherapy was 19 per cent (complete response 7 per cent; partial response 12 per cent) but only 2 per cent of all patients receiving chemotherapy had a pCR.

A single-institution trial from Queen Mary Hospital, Hong Kong randomized 147 patients with resectable oesophageal squamous carcinoma to two cycles of preoperative cisplatin and 5-FU chemotherapy or to surgery alone[46]. Although patients in the preoperative chemotherapy arm were more likely to have curative resection (67 *versus* 35 per cent), there was no significant difference in median survival between the two arms (17 *versus* 13 months respectively). The pCR rate was 7 per cent in the preoperative chemotherapy arm.

The Rotterdam Esophageal Tumor Study Group randomized 148 patients with resectable oesophageal squamous carcinoma to preoperative chemotherapy or to surgery alone[47]. Patients in the chemotherapy arm were reassessed after two cycles of cisplatin and etoposide; those who made a good initial response received a further two cycles before surgery and those who had an inadequate response proceeded immediately to operation. The median survival was longer in the chemotherapy arm (18 *versus* 11 months) but 2- and 3-year survival rates for the two arms are yet to be published.

The UK MRC recently completed a randomized trial (MRC OE02) of 802 patients with resectable oesophageal squamous carcinoma and adenocarcinoma comparing two cycles of cisplatin and 5-FU chemotherapy followed by surgery with surgery alone[48]. The early results indicate that patients receiving preoperative chemotherapy have a significantly higher curative resection rate (84 *versus* 71 per cent) and, consequently, an improved median (17·4 *versus* 13·4 months) and 2-year (45 *versus* 35 per cent) survival rate.

In summary, although preoperative chemotherapy results in clinical response rates of up to 70 per cent, pCR rates are relatively low. Results of two large randomized trials indicate a survival benefit[47,48], but two other trials indicate no benefit[45,46]. Although there is increasing evidence that preoperative chemotherapy may improve survival, a definitive conclusion from this generation of cisplatin-based neoadjuvant chemotherapy trials will come from further meta-analysis. The variation in results underlines the need to be able to define quality standards in patient selection, staging and treatment delivery.

Preoperative chemoradiotherapy

The use of synchronous chemotherapy with radiotherapy, otherwise known as chemoradiotherapy (CRT), is believed to increase the sensitivity of malignant cells to radiotherapy by impairing their ability to repair radiation-induced DNA damage. Early studies assessing the role of preoperative CRT in oesophageal cancer resulted in pCR in a significant proportion of patients treated[49–51], higher than would have been expected with chemotherapy or radiotherapy alone[37,38]. Subsequent studies have explored the role of preoperative CRT in improving local control, preventing distant metastasis and increasing overall survival in patients with oesophageal cancer. Its effects on surgical morbidity and mortality rates, on survival with and without pCR, and on the change in the pattern of relapse have also been documented. The observation of high pCR rates has led to randomized trials comparing preoperative CRT followed by surgery with surgery alone[34,52–56].

Results of non-randomized trials

There are at least 50 published trials of preoperative CRT in oesophageal cancer, most of which are single-arm, non-randomized studies. The 46 trials of both squamous carcinomas and adenocarcinomas, which contain over 20 patients are included in the summary shown in *Tables 1* and *2*[11,49–51,57–98]. Early trials used low radiotherapy doses (30 Gy) and later trials used higher doses (45–60 Gy), many of which involved unconventional fractionation schedules such as acceleration (over a shorter treatment duration) or hyperfractionation (treatment more than once a day). The most commonly used chemotherapy regimen was a combination of cisplatin and 5-FU in 38 trials, 12 of which also used a third agent (mitomycin C, etoposide, vinblastine, vincristine, hydroxyurea, interferon or paclitaxel). Six trials used a combination of mitomycin C and 5-FU. Most of the published trials report pCR rates, survival and pattern of relapse.

Owing to the small numbers of patients treated (range 22–182, median 46), data from individual trials on resectability, pCR and survival cover a wide standard deviation and are thus difficult to interpret. The pooled results of the 46 trials[11,49–51,57–98] (*Tables 1* and *2*) show that, of a total of 2704 patients (squamous carcinoma 69 per cent, adenocarcinoma 31 per cent) who received preoperative CRT, 2009 patients (74 per cent) had an oesophagectomy. One hundred and twenty patients (6 per cent) were found to have irresectable disease at surgical exploration. There were 643 pCRs, representing 24 per cent of the total number of patients treated or 32 per cent of those undergoing resection. Therefore, approximately one-quarter of all patients treated by preoperative CRT for oesophageal cancer, or one-third of those who have undergone oesophagectomy, will achieve pCR. What is the significance of pCR and its relationship to treatment outcome?

Response to preoperative chemoradiotherapy

pCR rates of 24–42 per cent were reported in resected patients treated in early trials of preoperative CRT using low radiotherapy doses (30 Gy) with synchronous chemotherapy[11,49–51,57–60]. In an attempt to increase the pCR rate, Urba *et al.*[65] in 1992 escalated the radiation dose to 49 Gy in 14 fractions over 3 weeks (3·5 Gy per fraction) with 5-FU chemotherapy in a trial of 24 patients. This caused considerable toxicity and failed to reproduce the results of earlier trials[66]; only two patients achieved pCR. In another trial of oesophageal adenocarcinoma, 30 patients received high-dose radiotherapy (60 Gy) with 5-FU and mitomycin C followed by surgery[72]. Seven patients had pCR, three patients died from treatment-related complications and the median survival was only 13 months.

Table 1 Non-randomized trials of preoperative chemoradiothreapy in oesophageal cancer

			Histology		Radiotherapy dose				
Reference	Year	No. of patients	SCC	Adeno	Gy	Fractions	Days	Gap (days)	Chemotherapy
Steiger *et al.*[49]	1981	86	86	0	30	15	19	No	5-FU, cispl or mito
Franklin *et al.*[50]	1983	30	30	0	30	15	21	No	5-FU, mito
Leichman *et al.*[51]	1984	51	51	0	30	15	21	No	5-FU, cispl or mito
Popp *et al.*[57]	1986	27	27	0	30	15	21	No	5-FU, cispl, ± vinc
Poplin *et al.*[58]	1987	113	113	0	30	15	19	No	5-FU, cispl
Seydel *et al.*[59]	1988	41	41	0	30	15	21	No	5-FU, cispl
Herskovic *et al.*[60]	1988	89	89	0	30	15	21	No	5-FU, cispl
MacFarlane *et al.*[11]	1988	22	9	13	30	15	19	No	5-FU, cispl, ± mito
Gignoux *et al.*[61]	1989	119	119	0	37	10	28	14	cispl (sequential)
Stewart *et al.*[62]	1989	29	25	4	45	23	31	No	5-FU, cispl, mito
Kavanagh *et al.*[63]	1992	103	77	26	45	25	33	No	cispl, 5-FU or etop
Naunheim *et al.*[64]	1992	47	29	18	30 or 36	15 or 20	21 or 28	No	5-FU, cispl
Urba *et al.*[65]	1992	24	0	24	49	14	21	No	5-FU
Forastiere *et al.*[66]	1993	43	22	21	38 or 45	15 or 30	21	No	5-FU, cispl, vinbl
Hoff *et al.*[67]	1993	68	29	39	30	15	21	No	5-FU/FA, cispl, ± etop
Sielezneff *et al.*[68]	1993	25	21	4	30	10	26	17	5-FU, cispl
Terz *et al.*[69]	1993	22	17	5	34	19	25	No	5-FU, cispl
Wolfe *et al.*[70]	1993	182	104	78	45	25	33	No	cispl, 5-FU or etop
Cordiano *et al.*[71]	1994	94	94	0	30	15	21	No	5-FU, cispl
Sauter *et al.*[72]	1994	30	0	30	60	30	42	No	5-FU, mito
Algan *et al.*[73]	1995	24	0	24	60	30	42	No	5-FU, mito
Girvin *et al.*[74]	1995	48	4	25	45	30	21	No	5-FU, cispl, vinbl
Vogel *et al.*[75]	1995	59	24	35	45	25	33	No	5-FU, cispl
Bates *et al.*[76]	1996	39	28	7	45	25	33	No	5-FU, cispl
Malhaire *et al.*[77]	1996	56	56	0	37	10	28	14	5-FU, cispl
Bessell *et al.*[78]	1996	53	53	0	36	15	21	No	5-FU, cispl
Chiappori *et al.*[79]	1996	87	36	51	30	15	21	No	5-FU/FA, cispl, etop
Stahl *et al.*[80]	1996	72	61	9	40	20	28	No	5-FU/FA, cispl, etop
Adelstein *et al.*[81]	1997	74	24	48	45	30	30	11	5-FU, cispl
Forastiere *et al.*[82]	1997	50	16	34	44	22	30	No	5-FU, cispl
Ganem *et al.*[83]	1997	88	82	6	40	20	35	7	5-FU, cispl, HU
Jones *et al.*[84]	1997	66	49	17	45	25	33	No	5-FU, cispl
Kane *et al.*[85]	1997	41	11	26	40 or 45	20 or 30	28 or 21	No	5-FU, cispl, IFN
Smithers *et al.*[86]	1997	90	58	32	35	15	21	No	5-FU, cispl
Suntharalingam *et al.*[87]	1997	32	16	16	50	28	38	No	5-FU, cispl
Thomas *et al.*[88]	1997	57	38	10	30	10	26	16	5-FU, cispl
Wright *et al.*[89]	1997	40	7	33	59	35	33	No	5-FU, cispl, paclitaxel
Adham *et al.*[90]	1998	55	55	0	36	12	16	No	5-FU, cispl
Bedenne *et al.*[91]	1998	96	96	0	30	10	26	16	5-FU, cispl
Keller *et al.*[92]	1998	46	0	46	60	30	42	No	5-FU, mito
Posner *et al.*[93]	1998	44	11	33	40 or 45	20 or 30	28 or 21	No	5-FU, cispl, ± IFN
Raoul *et al.*[94]	1998	32	32	0	45	30	19	No	5-FU/FA, cispl
Safran *et al.*[95]	1998	33	9	24	40	22	30	No	cispl, paclitaxel
Tamin *et al.*[96]	1998	51	8	43	30 or 45 or 54				5-FU, cispl
Gray *et al.*[97]	1999	73	21	49	45	25	35	No	carbo, paclitaxel
Iop *et al.*[98]	1999	53	48	4	43	25	33	10	5-FU, cispl
Total		2704	1826 (69)	834 (31)					

Values in parentheses are percentages. SCC, squamous cell carcinoma; Adeno, adenocarcinoma; 5-FU, 5-fluorouracil; cispl, cisplatin; mito, mitomycin C; vinc, vincristine; etop, etoposide; vinbl, vinblastine; FA, folinic acid; HU, hydroxyurea; IFN, interferon; carbo, carboplatin

Mandard *et al.*[99] in 1994 graded the degree of tumour regression found in the resected oesophagus of 93 patients who had received preoperative CRT. Tumour regression grade (TRG) was quantified from 1 to 5. TRG 1 represented fibrosis within the oesophageal wall with no identifiable residual cancer cells (pCR); TRG 2 represented rare residual cancer cells scattered throughout the fibrosis; TRG 3 represented predominant fibrosis with residual

Table 2 Non-randomized trials of preoperative chemoradiotherapy in oesophageal cancer

			pCR (%)		Mortality rate (%)		Survival (all)				Survival (pCR)			
Reference	Year	Resection (%)	All	Resection	CRT	Operative	Median (months)	2 years (%)	3 years (%)	5 years (%)	Median (months)	2 years (%)	3 years (%)	5 years (%)
Steiger *et al.*[49]	1981	56	17	31	11	10	8							
Franklin *et al.*[50]	1983	60	20	33	3	13	11				n.r.			
Leichman *et al.*[51]	1984	75	20	26	2	29	12							67
Popp *et al.*[57]	1986	37	24	33	15	17	9							
Poplin *et al.*[58]	1987	49	16	33	1	11	12	28	16		32	67	45	
Seydel *et al.*[59]	1988	66	20	30	5	4	13	15	8		15	33		
Herskovic *et al.*[60]	1988	56	14	24	n.s.	n.s.	10	20	10	6	30		33	
MacFarlane *et al.*[11]	1988	86	36	42	0	10		66	33				33	
Gignoux *et al.*[61]	1989	85	20	24	0	7								
Stewart *et al.*[62]	1989	59	17	29	7	6	10							
Kavanagh *et al.*[63]	1992	56	19	35	1	17	16	32	27					
Naunheim *et al.*[64]	1992	72	17	24	2	3	23	47	40		15	38	29	
Urba *et al.*[65]	1992	79	8	11	8	4	11	25	20					
Forastiere *et al.*[66]	1993	91	23	26	5	2	29	60	46	34	70	100	90	60
Hoff *et al.*[67]	1993	75	18	24	6	2	24	51	38					
Sielezneff *et al.*[68]	1993	84	28	33	0	8	18	22				35		
Terz *et al.*[69]	1993	77	23	29	5	12					18		40	20
Wolfe *et al.*[70]	1993	65	22	34	1	5					49			48
Cordiano *et al.*[71]	1994	70	16	23	0	10		34	23		32	51	40	40
Sauter *et al.*[72]	1994	60	23	39	7	5	13	33	25		23			
Algan *et al.*[73]	1995	50	17	33	4	8	15	40	28	28				
Girvin *et al.*[74]	1995	60	48	79	0	3					n.r.	65	65	
Vogel *et al.*[75]	1995	75	24	32	0	5		60	36	36		75	58	57
Bates *et al.*[76]	1996	90	46	51	0	9	22	47	36		37	61	61	
Malhaire *et al.*[77]	1996	79	38	48	0	11	37	65	55	30	40	70	70	30
Bessell *et al.*[78]	1996	100	25	25	0	15						92	92	69
Chiappori *et al.*[79]	1996	64	17	27	6	7	16							
Stahl *et al.*[80]	1996	67	22	33	6	15	17	36	33				68	
Adelstein *et al.*[81]	1997	88	24	28	1	18				49*				65*
Forastiere *et al.*[82]	1997	90	38	42	4	0	31	58			58	78		
Ganem *et al.*[83]	1997	58	22	37	6	11	18	42				78		
Jones *et al.*[84]	1997	82	33	41	0	11	19		32		25	55	45	
Kane *et al.*[85]	1997	90	24	27	7	5								
Smithers *et al.*[86]	1997	91	20	22	1	8	34	53	40	28				71
Suntharalingam *et al.*[87]	1997	100	31	31	n.s.	n.s.	19		41		n.r.			
Thomas *et al.*[88]	1997	84	21	36	n.s.	n.s.								
Wright *et al.*[89]	1997	90	35	39	5	5		61						
Adham *et al.*[90]	1998	96	42	43	0	7				27				53
Bedenne *et al.*[91]	1998	82	20	24	2	9	17	40	33	25				
Keller *et al.*[92]	1998	72	17	24	4	8	17	27			23			
Posner *et al.*[93]	1998	82	23	28	2	8	27	53	42		n.r.			
Raoul *et al.*[94]	1998	81	56	69	3	10		61	52					
Safran *et al.*[95]	1998	94	24	26	3	0								
Tamin *et al.*[96]	1998	100	29	29	0	8	16		38		18		53	
Gray *et al.*[97]	1999	77	41	54	0	10	24	50						
Iop *et al.*[98]	1999	92	21	22	4	10	36	58	47		42			
Mean		74	24	32	3	9								

*At 4 years. pCR, pathological complete response; CRT, chemoradiotherapy; n.s., not stated; n.r., not reached

cancer cells; TRG 4 represented residual cancer cells outgrowing fibrosis; and TRG 5 represented a complete absence of regression change. Univariate analysis showed that tumour size, pathological lymph node status, oesophageal wall involvement and TRG correlated with disease-free survival. However, only TRG (TRG 1–3 *versus* TRG 4–5) remained a significant predictor of disease-free survival on multivariate analysis.

Suntharalingam *et al.*[87] in 1997 performed surgical staging, including thoracoscopy and laparoscopy, in a series

Table 3 Pattern of recurrence following oesophageal resection after preoperative chemoradiotherapy

Reference	Year	No. resected	No. with recurrence Locoregional	Distant	Both	Total
Franklin *et al.*[50]	1983	18	4	8	1	13
Leichman *et al.*[51]	1984	23	1	9	2	12
Poplin *et al.*[58]	1987	55	2	9	0	11
Herskovic *et al.*[60]	1988	50	18	8	19	45
MacFarlane *et al.*[11]	1988	19	0	7	0	7
Kavanagh *et al.*[63]	1992	58	8	17	6	31
Urba *et al.*[65]	1992	19	3	6	0	9
Forastiere *et al.*[66]	1993	39	1	17	5	23
Hoff *et al.*[67]	1993	51	1	21	0	22
Sauter *et al.*[72]	1994	18	1	6	1	8
Algan *et al.*[73]	1995	12	2	4	1	7
Bates *et al.*[76]	1996	35	1	12	2	15
Malhaire *et al.*[77]	1996	44	17	9	0	26
Chiappori *et al.*[79]	1996	56	6	39	0	45
Stahl *et al.*[80]	1996	48	5	13	2	20
Adelstein *et al.*[81]	1997	65	1	17	0	18
Forastiere *et al.*[82]	1997	45	0	24	1	25
Ganem *et al.*[83]	1997	51	10	7	7	24
Jones *et al.*[84]	1997	54	0	15	2	17
Suntharalingam *et al.*[87]	1997	32	2	9	0	11
Wright *et al.*[89]	1997	36	0	2	0	2
Posner *et al.*[93]	1998	36	0	12	3	15
Raoul *et al.*[94]	1998	26	0	5	0	5
Total		890	83 (9)	276 (31)	52 (6)	411 (46)

Values in parentheses are percentages

of 32 patients with squamous cell carcinoma and adenocarcinoma before preoperative CRT. They found that 64 per cent of node-negative patients achieved pCR compared with none of the patients with evidence of node metastases. If these findings can be confirmed, the need for accurate staging will become of increased importance in future trials.

Effect on survival

Between 37 and 100 (mean 74) per cent of patients receiving preoperative CRT undergo oesophagectomy; the remainder are found to be unfit, irresectable or refuse operation following CRT. Of resected patients, 11–79 (mean 32) per cent achieved pCR. This represents 8–56 (mean 24) per cent of the total number of patients receiving preoperative CRT. Patients with pCR consistently survive longer than those who do not achieve pCR[55,56,66,74–76,78,80,82,84,87]. Their survival rate ranged from 29 to 92 per cent at 3 years and from 20 to 71 per cent at 5 years. However, some investigators noted no difference in survival between patients with pCR and those without[77,88], although deaths in the former group were exclusively from other causes (surgical complications, other cancers or cardiovascular events). Of those who did not achieve pCR, 23–33 per cent were alive at 3 years[11,66,76,78,84,96]. One trial documented that the only long-term survivors were those with pCR; those who did not achieve pCR all relapsed and died[51].

A few trials reported a number of long-term survivors (3-year survival rate 14–55 per cent) who received CRT but did not proceed to surgery, either because of patient refusal or poor operative risk[50,51,63,64,70,80,83]. However, some of these patients received further radiotherapy in addition to preoperative CRT.

Pattern of recurrence

Twenty-three trials involving 1256 patients reported the patterns of relapse following preoperative CRT and surgery (*Table 3*). Of 890 patients who had resection, 83 patients (9 per cent) had locoregional recurrence alone (range 0–39 per cent within individual trials). Distant metastases accounted for the majority of recurrences, occurring in 276 patients (31 per cent) (range 6–70 per cent within individual trials). Simultaneous locoregional and distant recurrences occurred in 52 patients (6 per cent). The overall risk of relapse was 46 per cent; the majority of relapses (80 per cent) were distant metastases or simultaneous locoregional and distant recurrences.

Table 4 Pattern of recurrence in patients with pathological complete response following preoperative chemoradiotherapy and oesophageal resection

			No. with recurrence			
Reference	Year	No. with pCR	Locoregional	Distant	Both	Total
Steiger *et al.*[49]	1981	15	0	0	0	0
Franklin *et al.*[50]	1983	6	0	1	0	1
Leichman *et al.*[51]	1984	6	0	1	0	1
Herskovic *et al.*[60]	1988	12	2	3	3	8
Gignoux *et al.*[61]	1989	24	0	2	0	2
Naunheim *et al.*[64]	1992	8	1	1	0	2
Sielezneff *et al.*[68]	1993	7	2	1	0	3
Terz *et al.*[69]	1993	5	0	3	0	3
Girvin *et al.*[74]	1995	23	0	8	0	8
Malhaire *et al.*[77]	1996	21	0	4	0	4
Jones *et al.*[84]	1997	22	0	3	1	4
Wright *et al.*[89]	1997	14	0	1	0	1
Posner *et al.*[93]	1998	10	0	0	0	0
Raoul *et al.*[94]	1998	18	0	1	0	1
Total		191	5 (3)	29 (15)	4 (2)	38 (20)

Values in parentheses are percentages. pCR, pathological complete response

Patients who achieved pCR had a significantly different pattern of relapse (*Table 4*). Fourteen trials reported the pattern of relapse in 191 patients who achieved pCR; five (3 per cent) had locoregional recurrence alone, 29 (15 per cent) had distant metastases alone and four (2 per cent) had simultaneous locoregional and distant recurrences. The overall risk of relapse was 20 per cent, mostly due to distant metastases.

Timing of surgery after chemoradiotherapy

The timing of surgery after CRT significantly affects the documented pathological response rate[66]. The overall pCR rate will be underestimated if surgery is scheduled before maximal response is attained and yet this interval is poorly documented in most trials. In rectal cancer, the Lyons R-90 trial[100] treated 210 patients by preoperative radiotherapy and randomized them to early (within 2 weeks) or late (6–8 weeks) operation. The preliminary results showed a higher pCR rate (15 *versus* 5 per cent) and a trend towards more restorative surgery in the late surgery arm, despite both arms receiving similar preoperative treatment. The distinction between pCR rather than residual microscopic disease could be a function of timing. If viable tumour cells are found within the resected oesophagus, does this imply treatment failure or were these cells about to undergo apoptosis? Shears *et al.*[101] examined residual tumour cells found in resection specimens for DNA nicks using the terminal deoxytransferase-mediated biotin–dexoyuridine triphosphate nick-end labelling assay to determine whether these cells were viable or undergoing apoptosis. Although apoptotic cells were identified within each specimen that contained residual tumour, they represented less than 10 per cent of the tumour cell total. It was concluded that the residual tumour cells found within the resected specimens were viable and would eventually lead to local failure.

The timing of surgery after chemotherapy or radiotherapy may also affect postoperative morbidity and mortality rates[102,103]. A period of time is necessary to allow the acute effects of treatment, including haematological and respiratory function, to recover completely before operation. If the potential for increased postoperative morbidity and mortality rates is to be avoided, the influence of timing should receive greater attention and emphasis in future studies.

Squamous cell carcinoma *versus* adenocarcinoma

Most of the early trials involved treatment of squamous carcinoma exclusively. However, more recent trials have included adenocarcinoma, a reflection of its steadily increasing incidence. Are these two distinct disease entities and should they be managed separately? The majority of adenocarcinomas arise around the gastro-oesophageal junction and only recently was distinction made between the various types of tumour at this site: lower oesophagus (type I), cardia (type II) and subcardial gastric (type III) tumour[104]. There is emerging evidence that adenocarcinomas of the lower oesophagus (type I) and cardia (type II) are a single clinical entity and should be renamed as gastro-

Table 5 Randomized trials of preoperative chemoradiotherapy and operation in resectable oesophageal cancer

			Histology		Radiotherapy dose								Overall survival			
Reference	Year	No. of patients	SCC	Adeno	Gy	Fractions	Days	Gap (days)	Chemotherapy	Resection (%)	pCR (%)	Operative mortality (%)	Median (months)	2 years (%)	3 years (%)	*P*
Nygaard *et al.*[34]	1992															
CRT + surgery		47	47	0	35	20	28	No	cispl, bleo*	66	n.s.	24	7	23	17	0·3
Chemo + surgery		50	50	0					cispl, bleo	58	n.s.	15	6	6	3	
Radio + surgery		48	48	0	35	20	28	No		54	n.s.	11	8	25	21	
Surgery only		41	41	0						68		13	6	13	9	
Le Prise *et al.*[52]	1994															
CRT + surgery		41	41	0	20	10	12	No	5-FU, cispl*	85	10	8	10	27	19	0·6
Surgery only		45	45	0						84		7	11	33	14	
Apinop *et al.*[53]	1994															
CRT + surgery		35	35	0	40	20	28	No	5-FU, cispl	74	20	12	10	30	26	0·4
Surgery only		34	34	0						100		15	7	23	20	
Walsh *et al.*[54]	1996															
CRT + surgery		58	0	58	40	15	21	No	5-FU, cispl	90	22	10	16	37	32	0·01
Surgery only		55	0	55						100		4	11	26	6	
Bosset *et al.*[55]	1997															
CRT + surgery		143	143	0	37	10	28	14	cispl*	78	20	12	19	48	36	0·8†
Surgery only		139	139	0						68		4	18	42	34	
Urba *et al.*[56]	1997	100	25	75												
CRT + surgery		50	n.s.	n.s.	45	30	19	No	5-FU, cispl, vinbl	n.s.	28	n.s.	17	41	32	0·04
Surgery only		50	n.s.	n.s.						n.s.		n.s.	18	36	15	

*Sequential. CRT, chemoradiotherapy; Chemo, chemotherapy; Radio, radiotherapy; SCC, squamous cell carcinoma; Adeno, adenocarcinoma; pCR, pathological complete response; cispl, cisplatin; bleo, bleomycin; 5-FU, 5-fluorouracil; vinbl, vinblastine; n.s., not stated. †Disease-free survival 40 *versus* 28 per cent at 3 years in favour of CRT + surgery ($P = 0{\cdot}003$)

oesophageal junction cancers[105,106] while subcardial tumours (type III) remain a separate entity.

How do squamous carcinoma and adenocarcinoma compare in terms of response to preoperative CRT and survival? Some trials detected no difference in pCR rate[11,67,75,84] while others found that patients with adenocarcinoma were less likely to achieve pCR[70,86]. Most trials found no difference in overall survival between those with squamous carcinoma and those with adenocarcinoma[66,70,75,80,82,84], but others reported conflicting results. Three trials found that patients with squamous carcinoma had a poorer survival[56,67,79], and two found a greater risk of recurrence following treatment of adenocarcinoma, hence a poorer survival[86,87]. Therefore, currently available evidence indicates that there is little or no difference in response rate and survival following preoperative CRT between squamous carcinoma and adenocarcinoma.

Conclusions from the non-randomized trials

Data from these non-randomized trials suggest that preoperative CRT significantly improves locoregional control in oesophageal cancer; most patients who relapse die from distant metastases rather than locoregional recurrence. However, does improved locoregional control translate into a survival benefit? What is the price of a combined modality approach in terms of additional morbidity and mortality? These questions can be answered only by randomized trials comparing this approach with surgery alone.

Results of randomized trials

There are presently six reported randomized trials comparing preoperative combined chemotherapy and radiotherapy followed by surgery with surgery alone in resectable oesophageal cancer[34,52–56] (*Table 5*).

The Second Scandinavian Trial[34] randomized 186 patients with squamous carcinoma to one of four arms: surgery alone, preoperative chemotherapy, preoperative radiotherapy, or preoperative chemotherapy and radiotherapy. Chemotherapy consisted of two cycles of cisplatin and bleomycin. Patients in the radiotherapy arms received 35 Gy in 20 fractions over 4 weeks, treating the entire oesophagus and mediastinum. Patients in the trimodality arm received two cycles of chemotherapy followed by radiotherapy sequentially. Although there was no overall survival difference between each of the four arms, the combined survival of all patients who had received radiotherapy was better than that of those who had not received radiotherapy (median 9 *versus* 7 months; 24 *versus* 10 per cent at 2 years).

Le Prise *et al.*[52] in 1994 reported a trial that randomized 86 patients with squamous carcinoma to two cycles of cisplatin and 5-FU followed by radiotherapy (20 Gy in ten fractions over 2 weeks) sequentially or to surgery alone. They found no difference in median (10 *versus* 11 months respectively) or 2-year (27 *versus* 33 per cent respectively) survival between the two arms.

Apinop *et al.*[53], also in 1994, reported a trial that randomized 69 patients with squamous carcinoma to preoperative CRT or surgery alone. CRT consisted of 40 Gy in 20 fractions over 4 weeks, with one cycle of synchronous cisplatin and 5-FU during the first week followed by a second cycle after completion of radiotherapy. There was no statistically significant difference in median survival between the two arms (10 *versus* 7 months respectively). However, seven of the 35 patients treated in the combined modality arm refused to have surgery following CRT.

Walsh *et al.*[54] in 1996 described 113 patients with adenocarcinoma who were randomized to preoperative CRT or surgery alone. Those in the CRT arm received 40 Gy in 15 fractions over 3 weeks, with one cycle of synchronous cisplatin and 5-FU during the first week followed by a second cycle after completion of radiotherapy. There was an overall survival benefit in favour of the CRT arm (median 16 *versus* 11 months; 32 *versus* 6 per cent at 3 years).

In the European Organization for Research and Treatment of Cancer (EORTC) trial[55], 282 patients were randomized to preoperative CRT or to surgery alone. The combined modality treatment consisted of two courses of radiotherapy (18·5 Gy in five fractions over 5 days) with cisplatin chemotherapy given 0–2 days before each radiotherapy course. The radiotherapy courses were separated by a 2-week interval. Patients receiving CRT were more likely to have curative resection (81 *versus* 67 per cent). Disease-free survival rate was significantly longer in the CRT arm (40 *versus* 28 per cent at 3 years) but there was no difference in overall survival rate (36 *versus* 34 per cent respectively at 3 years). This discrepancy was due to a substantially higher postoperative mortality rate in the CRT arm (12 per cent) than in the control arm (4 per cent), negating any overall survival benefit that may have been achieved by preoperative CRT.

The University of Michigan trial[56] randomized 100 patients with squamous and adenocarcinoma to preoperative CRT or to surgery (transhiatal oesophagectomy) alone. Patients in the CRT arm received 45 Gy in 30 fractions, given twice daily over 19 days with synchronous cisplatin, 5-FU and vinblastine. Although there was no difference in median or 2-year survival on initial analysis[107], a statistically significant difference in overall survival rate emerged at 3 years (32 *versus* 15 per cent) in favour of the CRT arm[56].

In summary, two of the randomized trials demonstrated that combined modality treatment improved overall survival[54,56]. A third trial demonstrated an improvement in disease-free survival but not in overall survival[55]. The remaining trials showed no difference in overall or disease-free survival[34,52,53]. These mixed and conflicting results may be explained by the considerable heterogeneity in patient variables (histology, stage and performance status) and in the treatment delivered (radiotherapy dose and fractionation, chemotherapy drugs, dose and scheduling). It is of note that only two trials used a conventional radiotherapy dose and fractionation of 35–40 Gy in 1·75–2·00 Gy per fraction[34,53]. Two trials used what may be considered a suboptimal radiotherapy dose (20 Gy)[52] or fractionation schedule (split course)[55]. In three trials[34,52,55] the chemotherapy and radiotherapy were given sequentially, and in the remaining three trials CRT was given synchronously[53,54,56].

Therefore, until the results of larger, well stratified, randomized trials become available, the role of preoperative CRT in resectable oesophageal cancer remains unresolved. Such trials are in progress in the USA and in Australasia[108].

Toxicity of chemoradiotherapy and surgery

What is the toxicity in terms of morbidity and mortality associated with this combined modality approach? Preoperative deaths were due to either CRT, progressive disease or a combination of both. Reported CRT-related deaths in the non-randomized trials ranged from 0 to 15 (mean 3) per cent (*Table 2*). Postoperative deaths ranged from 0 to 29 (mean 9) per cent of patients undergoing surgery. Adult respiratory distress syndrome, anastomotic leak and breakdown, pneumonia and sepsis were the commonest causes of death following oesophagectomy. Therefore, overall treatment-related deaths (CRT and postoperative) ranged from 3 to 25 (mean 9) per cent of all patients treated.

The risk of chemotherapy-related toxicity increases markedly with the number of chemotherapeutic agents used and increasing dose intensity of the CRT regimen[109–111]. Moderate to severe myelotoxicity was reported in 36–63 per cent of patients who received mitomycin C, vinblastine or etoposide in addition to 5-FU and cisplatin[62,66,67,80]. Those receiving etoposide as part of the preoperative CRT regimen were more likely to die from chemotherapy-related complications than those who did not[67].

Large-field radiotherapy to treat uninvolved nodes prophylactically[112] or large doses per fraction are also associated with toxicity[55,65]. The EORTC trial[55] reported a significantly higher postoperative mortality rate in the CRT arm, which was probably associated with the large radiotherapy dose per fraction (3·7 Gy per fraction) used. Similarly, Urba *et al.*[65] reported high complication rates in patients treated by high-dose radiation with synchronous chemotherapy using a large radiotherapy dose per fraction (3·5 Gy per fraction). Preoperative CRT may also increase the risk of developing a tracheobronchial fistula following oesophagectomy[113]. In contrast, Kane *et al.*[85] found that patients treated by preoperative CRT had shorter operation times and less operative blood loss than a historical surgical control group.

Is surgery essential?

Several investigators have demonstrated that definitive CRT alone in localized oesophageal cancer can result in survival rates similar to those of either surgery alone[58,112,114–117] or preoperative CRT followed by surgery[73,110,118]. Two randomized trials have demonstrated a significant survival advantage of CRT compared with radiotherapy[112,116,117]. Some clinicians have questioned whether there is any role for surgery in the primary management of oesophageal cancer[110,119]. Taking into account that the treatment-related mortality rate of combined CRT and surgery ranges between 5 and 10 per cent in high-quality units, the addition of surgery following CRT can only be justified if it still confers an improvement in overall survival when compared to patients treated by CRT alone. This question is being addressed by an ongoing EORTC trial (FFCD 9102) of locally advanced oesophageal cancer in which all patients receive induction CRT (30 Gy with synchronous cisplatin and 5-FU). Those assessed to have made a good initial response are then selected to be randomized to complete their treatment with either definitive CRT or surgery.

Until the results of the EORTC trial become available, the necessity of surgery can be speculated upon only by extrapolation of known results. First, although CRT is better than radiotherapy alone[112,116,117], many patients (40–60 per cent) still die from locoregional failure as defined by persistent disease or recurrence[111,112,118,120]. The reverse is true for patients treated by a combination of CRT and surgery; locoregional recurrence occurred in 9 per cent (*Table 3*) and most relapses involved systemic metastases. Second, patients who do not achieve pCR following preoperative CRT have a survival rate comparable to that of surgery or CRT alone (3-year survival rate 17–40 per cent; 5-year survival rate 15–32 per cent). One can only assume that if these patients had not undergone surgery, they would have died from locoregional recurrence[101].

Therefore, until CRT alone can consistently achieve high rates of locoregional control, surgical resection must remain an integral component of multimodality treatment in resectable oesophageal cancer. Furthermore, histological examination of the resected oesophagus remains the 'gold standard' means of determining response to CRT. Apart from pCR, microscopic residual disease within the resection specimen also predicts improved survival[99,121]. Future attempts to improve response rates to CRT can only be aided by the reliable and consistent means of assessment provided by surgical resection.

In the event of local failure following definitive CRT, a few trials have demonstrated that prolonged survival is possible with surgical salvage. Murakami *et al.*[122] described 40 patients treated by induction CRT; 30 who had made a good initial response were selected to complete their treatment with further radiotherapy. The remaining ten patients had surgical resection. The median survival was 45 months with no difference between the two treatment groups. Of the seven patients who developed local recurrence, four were salvaged surgically and three patients remained alive at 15–43 months. Algan *et al.*[73] described 35 patients with adenocarcinoma, 11 of whom were treated by by definitive CRT and 24 by CRT and surgery. Survival was similar in the two groups, with successful surgical salvage in two patients who initially received definitive CRT. Roca *et al.*[118] also reported a few patients who were salvaged successfully after local recurrence following definitive CRT.

Detecting pathological complete response

In view of the morbidity and mortality rates associated with oesophagectomy and the fact that CRT alone will be curative in a proportion of patients treated, is there a means of selecting patients who will not benefit from resection? They include those who will be 'cured' by CRT alone together with those who have occult metastatic disease and so are destined for early relapse regardless of the primary treatment modality. Patients who achieve pCR following preoperative CRT have an expected 5-year survival rate of approximately 60 per cent. They are most likely to have been 'cured' by CRT alone and so the additional benefit of surgery in this group must be questionable. How are these patients to be identified short of subjecting them to oesophagectomy?

The majority, 80–95 per cent, of patients treated by CRT will experience early improvement in swallowing function[123]. It is well known that, within a tube, a small increase in internal diameter results in a large reduction in flow resistance (Laplace's law), hence a minor response often leads to significant improvement in symptoms. It is hardly surprising than that symptomatic improvement after CRT does not correlate with the final pathological response[124,125].

The accuracy of barium studies and computed tomography (CT) in predicting pathological disease stage before preoperative CRT is poor[126] and these methods cannot be expected to be any better in assessing response to neoadjuvant treatment[127]. Endoscopic reassessment with mucosal biopsies after preoperative CRT is reported to be negative in 65–100 per cent of cases[50–52,58,75,76,84]. However, compared with the histology of the resected oesophagus, there is a poor correlation between endoscopic findings and pathological stage[121].

Role of endoscopic ultrasonography

Endoscopic ultrasonography can yield sufficient spatial resolution to distinguish the five layers of the oesophagus: mucosal surface, muscularis mucosa, submucosa, muscularis propria and adventitia. It is considered to be more accurate than CT in assessing the extent of local disease and nodal status of patients with untreated oesophageal cancer[126,128]. Findings consistently correlate with the pathological stage (both T and N stage) of the resected oesophagus with an accuracy of 80–90 per cent[128–131]. Its main limitation occurs when a severe stricture prevents intubation of the oesophagus. However, this situation in itself is recognized to be a poor indicator of prognosis, as such patients almost inevitably have locoregionally advanced disease[132,133]. The role of endoscopic ultrasonography in staging oesophageal cancer has been reviewed by Caletti *et al.*[134]. However, it is not widely available in the UK and its accuracy can be consistent only in experienced hands.

The use of endoscopic ultrasonography to assess response to neoadjuvant treatment in oesophageal cancer has also been well documented. Some investigators report a good correlation between sonographic findings and final pathological stage[135–137] but many others have failed to confirm this[138–144]. Restoration of normal oesophageal wall features on sonography seems to correspond to pCR[135,136], but such an appearance is not seen in every patient with pCR. The oesophageal wall often appears abnormal regardless of response to CRT and distinguishing residual tumour from treatment-related change on endoscopic ultrasonography is impossible. Giovannini *et al.*[136] found that some patients had discernible wall layers containing echo-poor nodules in the submucosa or muscularis propria following CRT, and correlated this appearance with either pCR or microscopic residual tumour in the resection specimen. These echo-poor areas probably represent fibrosis, inflammatory infiltrate and giant cell granulomas, which are commonly found following CRT[99]. In this study of 32 patients, sonography had 78 per cent sensitivity, 91 per cent specificity and 78 per cent positive predictive value in detecting pCR following preoperative CRT[136]. In a further study of 67 patients with irresectable oesophageal cancer treated by CRT alone, those who were found to have a complete response on endoscopic and sonographic examination survived significantly longer than those who had evidence of residual disease (49 *versus* 10 months)[145].

Role of endoscopic magnetic resonance imaging

Accurate staging of rectal and prostatic cancers can be achieved by endoscopic magnetic resonance imaging (MRI)[146,147]. In oesophageal cancer, the accuracy of endoscopic MRI in providing local and nodal staging information is similar to that achieved by endoscopic ultrasonography[148,149]. However, images were of inadequate quality to make an accurate assessment in a significant proportion of patients in one study[149]. Endoscopic MRI does offer some advantages over sonography; it is less operator dependent and images can be reconstructed in multiple planes to allow better conceptual visualization of the tumour. At the present time there are no published data on the use of endoscopic MRI in assessing the response of oesophageal cancer to neoadjuvant treatment.

Role of positron emission tomography

Instead of delineating anatomical structures, positron emission tomography (PET) is unique in its ability to visualize areas of increased metabolic activity within tissues. [^{18}F]Fluorodeoxyglucose is preferentially taken up by cells that have a high rate of glucose utilization (including brain, myocardium and most malignant cells). The ^{18}F radionuclide decays by positron emission, which can then be detected by the scanner. Comparison of PET with CT in oesophageal cancer[150–153] suggests that, although they are equally good at visualizing the primary tumour, they are equally poor at detecting involved perioesophageal nodes (28–76 per cent of involved nodes were identified correctly). However, an advantage of PET is its ability to detect distant metastases, which fail to be identified by CT. This occurred in 14–28 per cent of patients in studies in which PET findings were later confirmed by biopsy or by the subsequent development of disease at these sites[150–153].

There are few published data on the use of PET in assessing response to neoadjuvant treatment. In a study of

14 patients undergoing chemotherapy for oesophageal cancer[154] PET was no better than CT at determining response to treatment. With PET there is limited spatial resolution and possible attenuation of deep lesions, making the detection of small tumour lesions unreliable[150,152]. For this reason, PET is unlikely to become a useful tool in assessing response to neoadjuvant treatment. However, its routine use in conjunction with CT may be justified by its ability to spare patients with occult distant metastases an unnecessary major surgical procedure.

Conclusions and future approaches

The combination of chemotherapy with radiotherapy now has an established role in cancer treatment; it has replaced surgery as first-line therapy in anal cancer[155]. Early studies of preoperative CRT in anal cancer resulted in high pCR rates of 80 per cent[156,157]. Randomized trials have since confirmed the advantage of CRT alone as primary treatment instead of surgery[158,159]. CRT has also been shown to be superior to radiotherapy alone in the treatment of cervical cancer[160], non-small cell lung cancer[161], head and neck cancer[162], and oesophageal cancer[116]. In the National Cancer Institute PDQ Treatment Option Overview (www.cancernet.nci.nih.gov), CRT, with or without subsequent surgery, and surgery alone are listed as alternative standard treatment options for stage II and III oesophageal cancer. While not the subject of this review, there is accumulating evidence that CRT alone produces equivalent results, stage for stage, to surgery alone[163,164]. However, surgery remains the 'gold standard' against which new approaches must be compared and justified. In addition, an approach that includes resection allows the most accurate assessment of response following CRT treatment. Until consistently higher pCR rates can be achieved or there is a more accurate means of predicting pCR, there is justification for continuing with a policy of surgery following CRT in resectable oesophageal cancer, especially adenocarcinoma.

The results of treatment with surgery alone in early-stage disease are good[165]. However, patients more commonly present with locally advanced (if not metastatic) disease and so the overall survival figures for oesophageal cancer treated by surgery alone are less impressive[6]. It is essential for future studies to concentrate on accurate assessment of disease stage using modern imaging modalities, including sonography, spiral CT and MRI. Improvements in new modalities, such as PET, may further refine the selection of patients for complex therapies. Increasing morbidity and mortality rates by combining therapies is not justified for patients who, by accurate staging, would have either a good prognosis after surgery alone or a poor prognosis because of metastatic disease.

New treatment approaches for oesophageal cancer should reflect its potential for locoregional recurrence and metastatic disease. Proof of the link between improving locoregional control with radiotherapy and improving overall survival has been elusive, although there is strong evidence to recommend the combination of both radiotherapy and high-quality surgery in rectal[166] and breast[167] cancer. Effective systemic treatment is also required if an impact is to be made on survival related to metastatic disease. So far, cisplatin-based chemotherapy, alone or in addition to surgery, has produced no convincing evidence of improved systemic disease control. Combinations of cisplatin, continuous infusional 5-FU and radiotherapy are being explored. These have the advantage of using chemotherapy regimens closer to those with an impressive track record in the palliation of advanced disease[168]; they may also allow an improved interaction with radiotherapy[169]. New combinations including drugs such as paclitaxel are known to be active and have produced encouraging results[97].

Increased morbidity is a serious issue. There is convincing evidence that CRT, either alone or in combination with surgery, can produce high morbidity rates, and high dose per fraction radiotherapy seems to be a particular problem[55,65]. Optimal CRT combinations and high-quality delivery are essential to any routinely used strategy. Modern radiotherapy CT planning techniques with density correction, conformal beam shaping and a critical evaluation of target volume definitions should all contribute to lower morbidity. In addition to selecting patients for potentially curative therapy on the basis of stage, it is equally important to document performance status and comorbid conditions. Pretreatment assessment and nutritional support may have a significant impact on morbidity rates and tolerance to treatment. With changing demographics and an increasingly elderly population, comorbidity is an important determinant of outcome from all radical treatments.

Surgery alone is not without significant risk[170]. However, results are now being published by specialist units without excess morbidity or mortality. There is a learning curve for complex surgery[171] and it seems likely that similar factors apply to non-surgical treatments. Where complex chemotherapy, radiotherapy and surgery are to be combined, there is a need for the whole multidisciplinary team to gain both experience and expertise. There is evidence that a protocol-based approach to selection can improve the outcome of surgical treatment[20] and it is likely that a similar approach will allow patients to be selected for appropriate combined modality therapy.

In his 1999 review of multimodality therapy in squamous carcinoma of the oesophagus, Lehnert[172] questions the role of CRT. The very real problem of the variable quality control of surgery in reported series is also discussed. There are now consistent data to suggest that responders to preoperative CRT have a very different survival pattern from non-responders but, with confounding variables in the literature, it is difficult to be sure whether this survival advantage is conferred by treatment. The encouraging results from randomized and non-randomized trials presented in the present review suggest that there is an influence of treatment. It may be significant that the patterns of recurrence reported are altered compared with those associated with surgery alone. It is important that biological or other markers of response be identified for chemotherapy and radiotherapy to allow accurate selection for combined modality therapy as well as for radical non-surgical therapy[173,174]. For future studies it seems likely that pathways of care and treatment decisions for patients with squamous carcinoma and adenocarcinoma may diverge.

Data from new, larger, randomized trials, with quality control of the various components of selection, staging and treatment, may show the true value of preoperative CRT. Meta-analysis of existing randomized trial data will be helpful in guiding the direction of new work. Further international trials are underway and there are proposals for a new randomized trial in the UK. New models of patient management, with fewer, more specialized units, may improve the standards of treatment and outcome. They should also facilitate high-quality research as well as generate valuable audit data in this area of complex therapy.

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Randomized clinical trial of local bupivacaine perfusion *versus* parenteral morphine infusion for pain relief after laparotomy

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Background: Opioids are often used to decrease pain following laparotomy but are associated with unwanted side-effects. The effectiveness of local perfusion of bupivacaine 0·5 per cent following laparotomy was studied.

Methods: A prospective randomized study involving patients undergoing laparotomy for major colorectal surgery using a left iliac fossa skin crease incision was undertaken. Patients were randomized to receive either intermittent intravenous morphine infusion on demand with patient-controlled analgesia (PCA group) or continuous wound perfusion of local bupivacaine 0·5 per cent for 60 h (LA group).

Results: Seventy patients were recruited, 35 in each group. Patient demographics, surgical and recovery variables and complications were comparable in the two groups. The wound lengths were similar (median 14 cm in both groups). There was no statistically significant difference in postoperative pain scores at rest and with movement between the two groups, except for pain scores at rest on the first postoperative day ($P = 0·03$). The median total amount of morphine used was significantly greater in the PCA group (median 38 *versus* 0 mg in the LA group; $P < 0·001$).

Conclusion: Direct continuous local wound perfusion of bupivacaine 0·5 per cent is as effective as PCA for postoperative pain relief after laparotomy. It is a safe and feasible alternative to parenteral opioids.

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Introduction

Although parenteral opioids remain the mainstay for decreasing postoperative pain, they are associated with serious side-effects such as nausea, excessive sedation and respiratory depression. Although the dosage of opioids and associated side-effects can be reduced by use of a patient-controlled analgesia (PCA) system, these techniques are still associated with serious side-effects. Recently Partridge and Stabile[1] reported that long-acting local anaesthetics were highly effective for postoperative pain control. Wound infusion with local anaesthesia for the relief of postoperative pain has been shown to be useful previously[2–13].

The objective of this study was to explore the effect of direct continuous wound perfusion with bupivacaine 0·5 per cent on postoperative pain score, postoperative narcotic requirements and postoperative morbidity compared with patient-controlled opioid-based continuous infusion.

Patients and methods

A prospective randomized study was conducted from September 1999 to January 2000. Seventy consecutive patients (American Society of Anesthesiologists I–III with no history of hypersensitivity to bupivacaine or morphine), who underwent elective colectomy using a left iliac fossa skin crease incision, were recruited. They were randomized to receive either continuous subcutaneous wound infusion with bupivacaine 0·5 per cent via the ON-Q™ pain management system (Ethicon Endo-Surgery, Cincinnati, Ohio, USA) (LA group) or intermittent intravenous morphine infusion on demand with a PCA system (PCA group) for postoperative pain management.

All patients underwent standard colectomy with preoperative bowel preparation, prophylactic antibiotics and anticoagulation. All wounds were closed using a mass closure technique employing monofilament synthetic suture. Subcutaneous closure was not used and the skin was stapled. Patients in the PCA group received intravenous morphine for 60 h in amounts of 1–3 mg/h on demand via the PCA pump. The initial infusion rate was 1 mg/h. This was increased to a maximum of 3 mg/h if pain control was insufficient. For patients in the LA group a combination of 20 ml lignocaine 1 per cent and 20 ml bupivacaine 0·5 per cent was infiltrated into the peritoneum, muscles and subcutaneous tissue under direct vision before closure of the anterior abdominal wall. A small catheter was then inserted through a separate stab wound into the subcutaneous layer of the wound. This catheter was connected to the ON-Q$^{\text{TM}}$ pump, which was preloaded with 120 ml bupivacaine 0·5 per cent and infused continuously into the wound at a flow rate of 2 ml/h for 60 h. Subcutaneous morphine for breakthrough pain was given to patients in the LA group whenever requested.

Postoperative pain at rest and on movement was measured using a visual analogue scale (0 representing no pain and 10 the most severe pain) within 8 h of surgery and on the first, second and third days after operation. The amount of morphine used during the same period was also recorded. Other variables recorded included vital signs, sedation, confusional states, nausea, vomiting, chest infection, return of bowel function, time to postoperative ambulation, urinary retention, deep venous thrombosis and wound infection. All patients had a final wound examination at 4 weeks after operation.

Statistical analysis was performed using SPSS® (SPSS, Chicago, Illinois, USA). All data were examined for symmetry of distribution using stem and leaf plots. The variables were then compared and analysed using Fisher's exact test and Mann–Whitney U test. $P < 0{\cdot}05$ was deemed to be statistically significant.

Results

Thirty-five patients (median age 64 (range 15–83) years; 22 men) were randomized to the PCA group and 35 patients (median age 61 (range 41–82) years; 20 men) to the LA group. Patient demographics were similar between the PCA and LA groups.

The median incision length for both groups was similar: 14 (range 6–25) cm in the PCA group *versus* 14 (range 10–32) cm in the LA group. Nine patients in the PCA group and 13 patients in the LA group had a stoma created as part of the surgical procedure ($P = 0{\cdot}44$). Nine patients in the PCA group and 15 patients in the LA group had a drain inserted at the end of the operation ($P = 0{\cdot}21$).

Postoperative pain scores were measured using the visual analogue scale at rest and on movement within 8 h of surgery, and on the first, second and third days after operation. There was no statistically significant difference in pain scores between the two groups at rest and with movement, except in the scores experienced at rest on the first postoperative day ($P = 0{\cdot}03$). The median pain scores in the LA group were slightly higher than those in the PCA group on the day of operation but subsequently became identical. The median total amount of morphine used in the PCA group was significantly more than that in the LA group: 38 (range 12–181) *versus* 0 (range 0–20) mg ($P < 0{\cdot}001$) (*Table 1*).

Table 1 Postoperative pain scores and total morphine requirement

	LA	PCA	P*
Pain score			
At rest			
Day of operation	3 (0–8)	1 (0–8)	0·13
Day 1 after operation	2 (0–6)	0 (0–6)	0·03
Day 2 after operation	0 (0–4)	0 (0–6)	0·70
Day 3 after operation	0 (0–2)	0 (0–6)	0·61
During movement			
Day of operation	5 (0–10)	3 (0–10)	0·06
Day 1 after operation	3 (0–9)	3 (1–9)	0·17
Day 2 after operation	2 (0–7)	2 (1–8)	0·90
Day 3 after operation	1 (0–5)	1 (0–8)	0·88
Total morphine (mg)	0 (0–20)	38 (12–181)	<0·001

Values are median (range). LA, local anaesthesia; PCA, patient-controlled analgesia. *Fisher's exact test

None of the patients in either group was unduly sedated or confused owing to either form of analgesia during the study. Six patients in the PCA group compared with two patients in the LA group experienced vomiting ($P = 0{\cdot}26$). Six patients in the PCA group compared with five patients in the LA group developed low-grade fever. There was no significant difference in the time to return of bowel movement in the two groups: median time to passage of flatus after operation 2 (range 0–4) days in the PCA group compared with 2 (range 1– 4) days in the LA group. The median time to passing a bowel motion after operation was 3 (range 0–4) days in the PCA group compared with 3 (range 1–5) days in the LA group. None of the patients developed acute urinary retention after removal of the urinary catheter. The timing of postoperative mobilization was similar in both groups. No patient developed deep venous thrombosis.

Four patients in the LA group compared with one in the PCA group developed a wound infection ($P = 0{\cdot}36$). Patients were discharged from hospital a median of 5 (range 3–64) days after operation in the PCA group compared with 6 (range 4–18) days in the LA group ($P = 0{\cdot}42$).

Discussion

PCA with opioids is used widely for postoperative pain. The main disadvantage of parenteral opioids is their side-effects.

In this randomized clinical trial, the effectiveness of postoperative pain relief and the incidence of postoperative pain-related complications were compared in patients receiving direct wound infusion with local anaesthesia and PCA using opioid analgesia.

Skin crease incisions are thought by the present authors to result in less postoperative wound pain and have been used since the early 1990s to perform most major colorectal surgery. This study shows that the pain scores achieved using direct wound perfusion with bupivacaine 0·5 per cent are comparable to those achieved with PCA. Furthermore, only a minimal amount of morphine was needed in the LA group. In the present study, the catheter for perfusion of bupivacaine was inserted into the subcutaneous layer of the wound whereas other studies have reported catheter insertion between the rectal sheath and the peritoneum[2,6,8,9]. A mixed infiltration of long- and short-acting local anaesthesia (bupivacaine 0·5 per cent and lignocaine 1 per cent) was injected under direct vision into the peritoneum, muscle layer and subcutaneous fat before mass closure of the wound. In the LA group, 13 patients who had a stoma created and 15 patients who had a drain inserted had local anaesthesia infiltrated into the stoma or drain site at the end of the operation. Analgesia infusion was limited to the main wound alone. These patients neither experienced more pain nor demanded more 'rescue' morphine.

There is always a risk of overdose of bupivacaine from subcutaneous absorption, resulting in undesirable side-effects. In the present study the amount used was calculated to be below the maximum daily dose. No patient developed any untoward toxic effects. Reports in the literature show that the plasma concentration of bupivacaine after wound infiltration is well below the toxic threshold and may be reduced further when administered together with adrenaline[12,13].

Postoperative recovery was comparable between the LA and PCA groups. However, the total amount of morphine received by patients in the PCA group was much higher than that by patients in the LA group. One of the main attractions of the LA technique is the reduced (or absent) need for morphine for postoperative pain relief. The portability of the LA system used also allows easier mobilization than can be achieved with patients on parenteral opioids.

In conclusion, direct continuous subcutaneous wound perfusion with local anaesthesia provides effective postoperative pain relief in left lower quadrant skin crease laparotomy wounds. This technique is a feasible and safe alternative to patient-controlled morphine analgesia.

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Randomized clinical trial

Stoma-related complications are more frequent after transverse colostomy than loop ileostomy: a prospective randomized clinical trial

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Background: The consequences of leakage from low colorectal or coloanal anastomoses are reduced by the use of a loop stoma to divert the faecal stream. Controversy continues as to whether loop ileostomy (LI) or loop transverse colostomy (LTC) is the optimal method of defunctioning such anastomoses.
Methods: Patients requiring defunctioning following anterior resection and total mesorectal excision were randomized to receive either LI or LTC. Comparison was made between the groups regarding the difficulty of stoma formation and closure, the recovery after stoma closure and stoma-related complications. The minimum follow-up after stoma closure was 6 months (median 36 months).
Results: Between October 1995 and August 1999, 70 patients were randomized (LTC 36, LI 34) of whom 63 underwent stoma closure (LTC 31, LI 32). There were no significant differences in the difficulty of formation or closure, or in the postoperative recovery between the groups. However, there were ten complications related directly to the stoma in the LTC group: faecal fistula (one patient), prolapse (two), parastomal hernia (two) and incisional hernia during follow-up (five). None of these complications occurred in the LI group.
Conclusion: In this randomized study, the frequency of herniation before or after colostomy closure supports the choice of LI as a method of defunctioning a low anastomosis. Both methods appear to provide satisfactory protection for the low anastomosis.

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Introduction

Anastomotic leakage is a dreaded early complication of any intestinal anastomosis; there is a particularly high incidence following low colorectal and coloanal anastomosis[1]. The main factor determining the risk of leakage is the height of the anastomosis; the risk is particularly high when the anastomosis is less than 6 cm from the anal verge[1,2]. It is now generally acknowledged that a proximal defunctioning stoma does not abolish the risk of leakage, but certainly mitigates the consequences[1–3]. Dehni and colleagues[3] reported that the clinical leak rate was 17 per cent in patients who had a low colorectal anastomosis without a defunctioning stoma compared with 6 per cent in a similar group with a temporary stoma.

Loop stomas are commonly used to temporarily defunction distal colorectal or coloanal anastomoses, but there are complications associated with the stoma. While controversy continues as to whether all low anastomoses should have a temporary stoma[3] or whether selective usage is optimal[4], all agree that a stoma is occasionally necessary, and sometimes essential to prevent or treat the life-threatening complication of colorectal anastomotic leakage. In addition to moderating the consequences of anastomotic leakage, a temporary stoma may reduce the risk of complete anastomotic failure[3]. This is particularly relevant for low anterior resection (LAR) of the rectum, for which anastomotic leakage has been reported to be in excess of 10 per cent without a covering stoma[3,5]; consequent pelvic infection may result in a poor functional outcome or in anastomotic take-down and a permanent stoma[3,5].

Either a loop ileostomy (LI) or a loop transverse colostomy (LTC) can be used to defunction a low anastomosis, although there is no consensus as to the optimal temporary defunctioning stoma. Of the three published randomized controlled trials comparing loop stomas to defunction left-sided colonic anastomoses, one has favoured LTC[6] and two have recommended ileostomy[7,8].

This study was a prospective comparison of LTC and LI in a randomized controlled setting in a single institution in patients undergoing LAR and total mesorectal excision (TME).

Patients and methods

Only patients undergoing anterior resection and TME were considered for entry into this trial, which was approved by the research ethics committee. All patients had sites for LI and LTC marked before operation by a stoma therapist and informed consent was obtained. After the colorectal or coloanal anastomosis had been completed and a decision to create a defunctioning stoma made, the patient was assessed to confirm that either stoma was feasible. The patients were then randomized to receive either a LI or a LTC. The time taken to fashion the stoma, and the relative ease of this procedure was recorded using a personally designed linear scale.

After operation each patient was reviewed on a daily basis by the stoma therapist who prospectively assessed the daily stoma output. All complications related to the stoma before closure were recorded.

The stomas were closed in standard fashion, under general anaesthetic and with standardized antibiotic and venous thrombosis prophylaxis. The time taken to close the stoma and the relative ease of closure were recorded. The time to passage of first flatus and first faeces, and the postoperative stay were assessed. All complications following closure were noted.

All patients have remained under outpatient follow-up of their rectal neoplasms. During this follow-up the stoma sites are examined for the presence of an incisional hernia.

Data are expressed as median (range) and were compared using analysis of variance or χ^2 test with Yates' correction.

Results

Between October 1995 and August 1999, 115 anterior resections for rectal neoplasm were performed. Of these 16 patients had mesorectal transection for upper-third tumours and did not have a defunctioning stoma. Of the remaining 99 patients, 20 were defunctioned with a soft anal stent, five did not enter the study and the remaining 74 were considered for randomization. Four could not be randomized to LTC at operation owing to previous colonic resection, so 70 patients were randomized as shown in *Table 1*.

Table 1 Demographics

	LI (*n*=34)	LTC (*n*=36)
Age (years)*	63 (40–85)	68 (32–90)
Sex ratio (M : F)	27 : 7	22 : 14
Tumour height (cm)*	7 (3–12)	6 (3–15)
Tumour stage		
Benign	1	1
Dukes' A	4	12
Dukes' B	9	6
Dukes' C	16	11
Metastases	4	6

*Values are median (range). LI, loop ileostomy; LTC, loop transverse colostomy

Table 2 Time and ease of operation

	LI	LTC
Formation of stoma		
Time (min)	15 (10–30)	16 (5–30)
Ease*	3 (1–7)	3 (1–9)
Interval to closure (days)	62 (17–120)	73 (28–141)
Closure of stoma		
Time (min)	48 (40–105)	48 (25–90)
Ease*	7 (2–10)	4 (3–9)

Values are median (range). *Ease was measured on a linear scale from 1 to 10. LI, loop ileostomy; LTC, loop transverse colostomy

The loop stoma was closed in 63 patients, with a follow-up after closure of 36 (6–48) months. Five patients died before the stoma was closed; there were three postoperative deaths following anterior resection (two from myocardial infarction and one from mesenteric ischaemia), one patient died from advanced metastatic disease and one died following a stroke. Two patients required conversion to an end-colostomy following clinical leakage from the coloanal anastomosis, resulting in major pelvic sepsis.

In the LI group there was one clinical and one radiological leak, and in the LTC group there was one clinical leak. The overall leakage rate was three of 67.

There were no significant differences in time taken to create or close the stomas, or in the difficulty of the procedure (*Table 2*). The postoperative recovery times following stoma closure were similar for both groups of patients (*Table 3*). At closure bowel resection was required in two patients who underwent LTC compared with six who had a LI.

There were no deaths attributable to a stoma or following stoma closure. The complications related to stomas and postoperative morbidity following stoma closure are shown

Table 3 Recovery from stoma closure

	LI	LTC
Time to first flatus (days)	2 (1–5)	2 (1–5)
Time to first defaecation (days)	3 (1–6)	4 (1–6)
Time to hospital discharge (days)	6 (4–13)	6 (4–9)

Values are median (range). LI, loop ileostomy; LTC, loop transverse colostomy

Table 4 Stoma- and stoma closure-related complications

	LI	LTC
Directly-related complications		
Stoma prolapse	0	2
Parastomal hernia	0	2
Faecal fistula	0	1
Incisional hernia	0	5
High-output stoma	1	0
Total	1	10
Other complications		
Wound infection	1	2
Small bowel obstruction	0	1
Deep venous thrombosis	1	1
Pulmonary embolus	1	0
Urinary retention	1	0
Total	4	4

LI, loop ileostomy; LTC, loop transverse colostomy

in *Table 4*. There were more complications in the LTC group than in the LI group (χ^2 = 4·46, 1 d.f., P = 0·05). The difference was accounted for by direct colostomy-related complications in the LTC group (*Table 4*).

Discussion

Low colorectal and coloanal anastomoses have the highest risk of leakage of any intestinal anastomosis[1–3,5]. Patients undergoing LAR with TME were selected for this study as the leak rate is among the highest for elective colorectal or coloanal anastomoses[5]. Anastomotic leakage may be reduced by triple stapling[1] and the routine use of a stapled colon pouch[9].

Three previous trials have addressed the question of whether a LI or LTC is the optimal stoma for defunctioning a colonic or colorectal anastomosis. Williams and colleagues[7] reported 47 patients (LI 23, LTC 24) who underwent elective colorectal surgery. They found significant differences in favour of LI with regard to odour and appliance changes, and a higher wound infection rate associated with LTC closure. Khoury and colleagues[8] recruited 61 patients (LI 32, LTC 29) into their trial, of which 52 had closure of the stoma. They found no significant difference between the groups, except that LIs functioned earlier than LTCs. They recommended ileostomy as an alternative to LTC. The third study, that of Gooszen and colleagues[6], contained 76 patients (LI 37, LTC 39) and reported statistically better results with LTC as a temporary defunctioning stoma. However, this was a multicentre trial in five centres over a period of 6 years. Patients presenting as an emergency with left-sided colonic obstruction were included with those having elective surgery for any colorectal pathology, including sigmoid diverticular resection.

In the present series there was no significant difference in the ease of construction and the time taken to fashion LIs and LTCs although, as suggested by Khoury and colleagues[8], LIs appeared to be more difficult to construct in the markedly obese. In contrast to the findings of Williams and colleagues[7], there was no difference in complications related to management of the different stomas in the current study. This may be due to improvements in stoma appliances and accessories that have resulted in a reduction in seepage of liquid faeces from a flush LTC and in the odour associated with LTC (a major factor in previous series).

In this series consultants or senior trainees performed stoma closure. There were no significant differences in the time taken to close stomas, or in the ease of the procedures. However, LI closure did appear more difficult than LTC closure, perhaps because of the need to either reduce or resect the spout of the ileostomy (six LIs *versus* two LTCs). In addition, the fascial defect used to create LTCs is larger than that for LIs, improving access to the peritoneal cavity for stoma mobilization at the time of closure. It may be as a result of the relative bulk of a loop colostomy compared with a LI and the size of the fascial opening that two stomal prolapses and two parastomal hernias were observed in the LTC group.

The postoperative recovery from stomal closure was similar for LI and LTC. However, during follow-up five patients developed an incisional hernia at the site of the LTC. This cannot be explained by the difficulty of closure, as LTCs appeared somewhat easier to close than LIs. The higher bacterial concentration of LTC effluent may have resulted in greater contamination of the wounds at the time of closure, increasing the risk of deep wound dehiscence. Alternatively, the spout of an ileostomy may reduce leakage of faecal fluid during mobilization of the ileostomy. Whatever the mechanisms, the frequency of LTC complications both before and after closure resulted in a significantly greater number of revisional procedures in the LTC group than the LI group.

LI may be associated with a high incidence of adhesion-related small bowel obstruction following total colectomy and an ileoanal pouch procedure, but after distal colorectal resection this complication is seen less frequently[10]. There are concerns that with longer follow-up adhesion-related complications might become more evident, particularly after LI. To date, however, there has been only one patient (in the LTC group) with small bowel obstruction. Longer follow-up may also result in detection of further incisional hernias, seen more frequently in the LTC group.

This prospective randomized trial has shown a significantly greater number of major stoma-related complications associated with LTC. Both methods appeared to provide effective defunctioning of the high-risk anastomosis and the overall leak rate of 4 per cent (three of 67) is among the lowest reported in a series of patients with TME and low anastomoses. The high incidence of stoma-related complications supports the use of LI as the optimal defunctioning stoma for patients who require a stoma to defunction a low anastomosis following LAR and TME with adequate bowel preparation. A loop colostomy may provide better defunctioning in a poorly prepared bowel, or where leakage has occurred and defunctioning is required.

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Original article

Treatment strategy for patients with middle and lower third bile duct cancer

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Background: The prognosis for patients with middle and lower third bile duct carcinoma remains poor. This study was conducted to identify independent predictors for survival, as well as the patterns of recurrence after curative resection.

Methods: Sixty-seven patients with pathologically verified middle and/or lower third bile duct carcinoma were analysed retrospectively by Cox regression analysis for predictors of survival.

Results: The overall 5-year survival rate after resection was 39 per cent, and 0 per cent for patients who did not undergo resection. The 5-year survival rate was 63 per cent in 26 patients without microscopic residual disease (R_0), 16 per cent in 25 patients with microscopic residual tumour (R_1) and 0 per cent in six patients with macroscopic residual tumour (R_2); ten patients did not undergo resection. Radiotherapy improved the 5-year survival rate in eight patients who had R_1 resection compared with the rate in 17 patients who underwent resection alone (8 *versus* 0), but not significantly so ($P = 0{\cdot}137$); however, median survival was significantly longer ($P = 0{\cdot}004$) in six patients who had R_2 resection compared with that in ten inoperable patients (11·4 *versus* 3·5 months). Multivariate analysis revealed that the primary tumour and tumour node metastasis (TNM) stage were independent predictors of survival; 13 clinicopathological factors were not independent prognostic factors. Of 26 patients having R_0 resection, one had a locoregional relapse only, six had distant metastases only, and five had both types of recurrence. The liver was the most frequent site for metastasis, and microscopic venous invasion (MVI) in the primary tumour was a significant predictor of liver metastasis.

Conclusion: Curative (R_0) resection is only one step in curing cancer, and radiotherapy may play a beneficial role in controlling locoregional residual tumour. MVI could be a useful indicator of when systemic adjuvant therapy should be implemented to prevent liver metastasis after R_0 resection, although no effective systemic treatment has yet been developed.

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Introduction

Even in recent reports, the 5-year survival rate after resection of middle–distal bile duct carcinoma remains low (at around 40 per cent), despite resectability rates as high as 90 per cent[1,2]. This discrepancy between the resection rate and long-term survival suggests the need to optimize treatment strategies in order to improve the prognosis. With this goal in mind, the identification of independent prognostic factors and patterns of relapse would be useful in such patients. However, these factors have not yet been fully identified because of the rarity of the disease and difficulty in excluding patients with similar carcinomas that arise from both the papilla of Vater and the head of the pancreas.

This retrospective study was undertaken to identify the clinicopathological factors that significantly influence long-term survival and to define the pattern of relapse after curative resection. In addition, the study aimed to determine the predictors of relapse in patients undergoing curative resection for pathologically confirmed middle and lower bile duct carcinoma. The efficacy of radiotherapy for patients with this disease was also examined and the implications of incorporating radiotherapy as a component of multidisciplinary treatment were considered.

Patients and methods

Sixty-seven consecutive patients with a pathological diagnosis of middle to lower third bile duct carcinoma treated between October 1977 and June 2000 were reviewed retrospectively. The anatomical locations of the tumours were categorized into three groups based on macroscopic and microscopic findings, and defined according to Japanese general rules for surgical and pathological studies on cancer of the biliary tract[3]. That is, the upper duct was defined as the cephalic portion of the bisected bile duct between the confluence of the bilateral main hepatic ducts and the level of the superior edge of the pancreas. The middle duct was defined as the caudate portion of the bisected duct. The distal bile duct begins from the entrance of the pancreas to the entrance of the duodenal wall.

Of 67 patients, 57 underwent surgical resection (resectability rate 85 per cent), and ten did not have tumour resection because of advanced disease or poor medical condition. All 67 patients were followed for a mean of 42 (range 0·6–216) months. Men ($n = 50$) outnumbered women ($n = 17$) by a ratio of 2·9 : 1; the mean age was 65 (range 32–85) years. The tumours were classified by the tumour node metastasis (TNM) classification criteria[4]. Of the 67 patients, 64 (96 per cent) had hyperbilirubinaemia on admission and the preoperative (or prebile drainage) maximum total serum bilirubin level ranged from 32·5 to 670 (mean 241) μmol/l. Percutaneous transhepatic biliary drainage (PTBD) was performed to relieve jaundice in 62 patients with a mean preoperative maximum serum bilirubin level of 250 (range 43–694) μmol/l. Resections were conducted after PTBD in 52 patients. All resections were performed following routine diagnostic imaging consisting of ultrasonography, cholangiography, computed tomography and angiography.

Resection procedures and indications

Hepaticocholedochus resection (HCDR), defined as a bile duct resection of the right and left hepatic ducts or of the common hepatic duct, was conducted in 38 and 19 patients respectively. In addition to hepatic bile duct resection, pancreaticoduodenectomy (PD) was conducted in 49 patients, and pylorus-preserving pancreaticoduodenectomy (PPPD) or total pancreatectomy was performed in one patient for each procedure. Thus, in six patients HCDR alone was performed. PD was carried out when the main tumour and/or tumour extension were on the intrapancreatic common bile duct. HCDR was performed when the tumour approached the confluence of the main hepatic ducts or common hepatic duct. PPPD was undertaken when the tumour was located in the intrapancreatic common bile duct and was of the polypoid or nodular type, as these types of tumour do not usually penetrate deep into the wall of the bile duct[5]. Total pancreatectomy was performed when lymph node metastases were beyond the scope of PD. All lymph nodes in the hepatoduodenal ligament, periduodenal area, around the common hepatic artery, and on the right side of the coeliac artery were dissected for resections of curative intent. In addition, nodes of the periportal region, the right side of the superior mesenteric artery, and the right side of the intra-abdominal aorta from the superior margin of the coeliac trunk to the superior margin of the inferior mesenteric artery, were routinely dissected for patients with swollen N_2 nodes. The portal vein of ten patients was reconstructed following resection.

Radiotherapy adjuvant to surgical resection

Radiotherapy was performed in 15 patients. Ten patients were treated by external-beam irradiation for 4–6 weeks after PD resection with a mean total dose of 47·2 (range 30·6–59·4) Gy, using a fraction size of 1·8–2·0 Gy. The hepaticojejunostomy, portal vein, hepatic arteries, coeliac and superior mesenteric arteries were included within the radiation field. Intraoperative radiation therapy was administered to three patients using a single dose of 25 or 30 Gy after PD[6].

Two patients with irresectable tumours were treated by external-beam radiotherapy following insertion of an expandable metallic stent through the bile duct after recanalization by percutaneous endoscopic microwave tumour ablation[7]. One of these two patients had bone metastases; the primary tumour and lymph node status were T_3 and N_2 respectively. In the other patient, the lesion was estimated as stage III ($T_2 N_0 M_0$); however, resection was refused because of high-risk factors for PD: age (80 years), diabetes mellitus, hypertension and a past history of myocardial infarction. Pathological diagnosis was confirmed by biopsy through percutaneous biliary endoscopy, and the disease stage was determined by diagnostic imaging.

Statistical analysis

Survival curves were calculated according to the Kaplan–Meier method and comparisons were made with the log rank test. The prognostic significance of each clinicopathological variable was calculated in the subpopulation of 52 resected patients with adenocarcinoma, after the exclusion of two patients who had died by postoperative day 45.

Univariate and multivariate analyses carried out by means of the Cox proportional hazard model were performed in order to determine the prognostic factors for survival. With regard to the multivariate Cox regression, the TNM stage grouping and its defining factors (T, N and M categories) were not used simultaneously because of the high correlation coefficients. The correlation matrix of explanatory variables was examined with the Pearson correlation coefficient[8]. $P < 0{\cdot}05$ was considered statistically significant.

Results

Tumour location, histopathology and tumour node metastasis stage grouping

Twenty-eight of the lesions were located in the lower third of the common bile duct, including seven lesions that extended to the middle third of the common duct. There were 16 lesions situated mainly in the middle third of the duct, including eight that extended to the lower third of the common bile duct and three that invaded the upper third of the bile duct (common hepatic duct). In the remaining 13 patients the main tumour was at the middle to lower third, extending to the upper third of the bile duct.

Adenocarcinoma was the most frequently noted histopathological type (59 patients; 88 per cent), followed by adenosquamous carcinoma (two patients; 3 per cent) and squamous cell carcinoma (one patient; 1 per cent). In the remaining five patients, the histopathological type was not defined because of insufficient sampling material. The histological grade in 54 resected patients with adenocarcinoma was classified according to TNM criteria[4]; 32 patients had well differentiated (G_1), 15 had moderately differentiated (G_2) and seven had poorly differentiated (G_3) tumours, but no patient had an undifferentiated (G_4) tumour.

Of the 57 resected patients, eight had stage I, five had stage II, eight had stage III, 26 had stage IVA and ten had stage IVB disease. TNM staging is shown in *Table 1*.

Morbidity, mortality and survival

Perioperative death occurred in three patients between days 2 and 45 after operation. Major complications occurred in seven resected patients and included hepatic infarction, bleeding from the stump of the gastroduodenal artery, gastrointestinal bleeding, intra-abdominal abscess and leakage of the bilioenteric anastomosis in one case each, and two pancreatic fistulas.

Of ten unresected patients (four with stage IVA, four with stage IVB and two with stage III disease), none has survived for more than 21·4 months after biliary drainage. The overall(s.e.) 1-year survival rate and median(s.e.) survival of the ten patients were 20·0(12·6) per cent and 4·9(3·2) months respectively.

Fifty-one patients had a radical resection and six had palliative resections because of their poor medical condition. Twenty-six patients had a curative (R_0) resection, which was defined as having no residual microscopic tumour after resection, no cancer cell invasion in the surgical margins, and all lymph node metastases restricted to the scope of the dissection. Although gross tumour was resected completely in 25 patients, microscopic involvement of the surgical margin (R_1) was discovered after operation. In six patients, tumour invasion was visible in the surgical margin (R_2). Cancer cells were most frequently present at the radial ductal margins in R_1 resections. Microscopic invasion was also revealed in the cut edge of the hepatic duct. The incidence of cancer cell invasion at the above two sites was 22 of 25 and eight of 25.

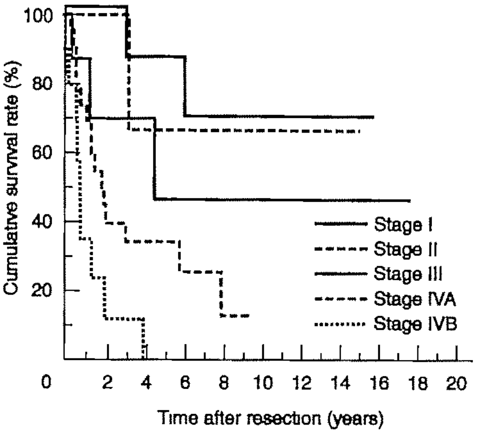
At 30 June 2000, 14 of the 57 resected patients were alive; the overall(s.e.) 5-year survival rate and median(s.e.) survival time including perioperative deaths were 39·1(7·5) per cent and 37·1(17·9) months respectively. The 5-year survival rate decreased with stage: 86, 67, 49, 34 and 0 per cent for stages I, II, III, IVA and IVB respectively (*Fig. 1*). It was also decreased with R status: 63, 16 and 0 per cent for R_0, R_1 and R_2 respectively (*Fig. 2*). Thirteen patients have survived more than 5 years, four of whom had stage IVA, two had stage III, one had stage II and six had stage I disease. Of the 5-year survivors, 12 patients had R_0 disease, including one patient who received intraoperative electron beam irradiation, whereas one patient (stage IVA) with R_1 disease received postoperative radiotherapy.

Survival after adjuvant radiotherapy improved in eight patients with R_1 disease, but was not statistically significant ($P = 0{\cdot}137$) compared with survival in 17 patients undergoing resection alone. The 5-year survival rate and median survival were 51 per cent and 98·2 months respectively in

Table 1 Tumour node metastasis stage grouping of resected patients

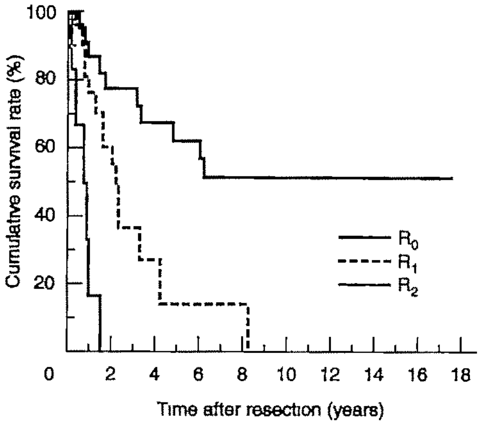
	n	N_0M_0	N_1M_0	N_2M_0	N_1M_1	N_2M_1
T stage						
T_1	11	8*	2‡	1‡	0¶	0¶
T_2	12	5†	3‡	2‡	1¶	1¶ (1)
T_3	34	8§	9§	9§	0¶	8¶ (6)
Total	57	21	14	12	1	9

Values in parentheses are the number of M_1 tumours due to periaortal node metastasis. Lymph node metastasis beyond N_2 was classified as M_1.*Stage I; †stage II; ‡stage III; §stage IVA; ¶stage IVB



No at risk							
I	8	8	7	7	6	6	1
II	5	3	3	3	2	1	1
III	8	6	4	4	3	1	1
IVA	26	17	10	7	4	4	1
IVB	10	3	2	1	1	0	0

Fig. 1 Kaplan–Meier survival curves after resection of middle and lower third bile duct cancer in accordance with tumour node metastasis stage grouping. $P = 0.0004$ (log rank test)



No at risk							
R_0	26	20	16	16	13	12	5
R_1	25	16	10	6	2	1	0
R_2	6	1	0	0	0	0	0

Fig. 2 Kaplan–Meier survival curves in patients with residual tumour. R_0, no cancer cell residue; R_1, microscopic residue; R_2, macroscopic residue. $P < 0.0001$ (log rank test)

the radiation group, and there were no 5-year survivors in the resection-alone group. The survival of patients with macroscopic disease was prolonged by radiotherapy. Median survival was 11·4 months after radiotherapy for six patients with macroscopic disease (four patients with R_2 disease and two with an irresectable tumour). On the other hand, median survival was only 3·5 months for ten patients who did not have radiotherapy (R_2, two patients; irresectable tumour, eight) ($P = 0.004$).

Predictors of survival

Univariate analysis revealed that age (70 years or more; $P = 0.015$), sex ($P = 0.0031$) and success of tumour resection (resectable *versus* irresectable; $P < 0.0001$) were significant predictors for survival. Only three patients had a normal preoperative serum bilirubin level (less than 17·1 µmol/l), two of whom survived for 5 years. However, the presence of jaundice did not influence survival, nor did the mean preoperative maximum bilirubin level (241 µmol/l or greater) correlate with survival. The cut-off value (37 units/dl or more) of the serum carbohydrate antigen (CA) 19-9 level did not serve as a significant survival predictor, excluding 14 patients whose CA19-9 level was not assessed.

The prognostic significance of each clinicopathological variable following resection was calculated in the 52 patients with adenocarcinoma after exclusion of two perioperative deaths. Sex, T, N and M categories significantly influenced survival, as well as TNM stage, histological grade (G), microscopic venous invasion (MVI) of the primary tumour and R status (*Table 2*). On the other hand, age, jaundice, CA19-9 level, tumour location, perineural invasion and lymph vessel invasion were not significant predictors of survival.

Only T (risk ratio 5·620) and TNM stage (risk ratio 6·681) were identified as independent factors influencing survival as determined by multivariate analysis (*Table 3*).

Patterns of recurrence and predictors of metastasis

Nine of 24 patients who had an R_0 resection, excluding two perioperative deaths, continued to live after 3·8–18 years without evidence of recurrence. However, recurrence occurred in 12 patients between 6 months and 9·6 years after resection. Three patients died from unrelated disease (not cancer) between 3·1 and 190·2 months after PD. After R_0 resection only one patient developed locoregional failure alone; six patients had distant metastasis alone, and five developed both types of failure. The overall frequency of distant metastasis was 11 of 24, and that of locoregional failure was six of 24. Different modes of recurrence were identified, but all distant metastases occurred in the liver, whereas the second most common sites of metastasis were the peritoneum (four of 11) and distant lymph nodes (four of 11). Pulmonary metastasis occurred in only one patient.

Fisher's exact test revealed that the presence of MVI in the primary tumour correlated significantly ($P = 0.033$) with the development of liver metastasis after curative resection of the middle–distal bile duct tumour. However, no significant correlation was demonstrated between the postoperative development of liver metastasis and lymphatic or perineural invasion (*Table 4*).

Table 2 Prognostic factors related to survival after resection in 52 patients* (univariate analysis)

Prognostic factor	No. of patients	5-year survival rate (%)	Median survival (months)	*P*§
Sex				
F	11	18	14·9	0·032
M	41	41	38·8	
Primary tumour (T)				
T_1	11	89	—	0·0003
T_2	11	29	50·0	
T_3	30	20	18·2	
Lymph node involvement (N)				
N_0	17	61	—	0·011
N_1	15	41	26·1	
N_2	20	9	18·2	
Distant metastasis (M)				
M_0	42	45	39·1	0·0003
M_1	10	11†	11·2	
TNM stage				
I	8	86	—	0·0001
II	4	67	—	
III	8	47	56·6	
IVA	22	27	23·9	
IVB	10	11†	11·2	
Histological grade (G)				
G_1	31	53	71·7	0·0056
G_2	15	24	19·6	
G_3	6	17†	11·2	
Microscopic venous invasion				
Negative	22	65		0·001
Positive	30	16	18·6	
Residual tumour (R)				
R_0	22	59	73·4	0·0001
R_1	24	15	26·1	
R_2	6	17‡	9·1	

*Five patients excluded because of adenosquamous or squamous cell carcinoma, or death within 45 days after operation; †4-year survival rate; ‡1-year survival rate. §Log rank test

Discussion

Lymph node involvement (N)[1,2,9,10], surgical margin or residual tumour (R)[1,9–11] and histological grade (G)[1,9,11] have been identified by multivariate analysis as independent predictors of survival after surgical resection of middle and distal bile duct cancers. Several other parameters, such as age[2], MVI[2], performance status[11], total bilirubin level[11] and tumour size[9], were also identified as independent factors influencing survival in multivariate analysis, although these have not been observed consistently in all studies. In this study, T and TNM stage were independent factors, but sex, N, R, G and MVI were not, despite univariate analysis revealing them to be significant prognostic factors. Significant multiple co-linearity among explanatory variables might have influenced the outcome of multivariate analysis in this study. Examination of the correlation matrix revealed that Pearson correlation coefficients were 0·442 between T and N ($P = 0{\cdot}001$), 0·414 between T and R ($P = 0{\cdot}0023$), 0·510 between T and MVI ($P = 0{\cdot}0001$) and 0·394 between M and G ($P = 0{\cdot}0039$). Univariate as well as multivariate analysis did not show perineural invasion to be a significant independent survival indicator, despite Bhuiya *et al.*[12] demonstrating it to be so by multivariate analysis. Tompkins *et al.*[13] reported that patients with middle third bile duct cancers have a poorer prognosis than those with lower third disease; however, the present study did not indicate a significant difference in survival by tumour location, similar to the findings of another recent report[10].

Surgical resection provides the only chance of cure, and recently reported operative mortality rates (less than 5 per cent) have established pancreaticoduodenectomy as a standard surgical treatment. Despite resectability rates that exceed 90 per cent, the overall 5-year survival rates remain low at around 30 per cent[1,2]. This discrepancy between resectability and long-term survival rates might be a reflection of the advanced stage of the disease. The present data clearly demonstrate that R_0 resection provided a potential cure (5-year survival) for 63 per cent of patients ($n = 26$). This rate was significantly decreased to 16 per cent when resection was complete at the macroscopic level but microscopic tumour residue remained (R_1).

Table 3 Significant risk factors affecting survival following resection (Cox multivariate analysis)

Model*	Variable	β coefficient	Standard error	χ^2	*P*	Risk ratio
1	Primary tumour (T)	1·723	0·848	4·144	0·0418	5·620 (1·066–29·622)
2	TNM stage	1·899	0·947	4·024	0·0448	6·681 (1·045–42·731)

Values in parentheses are 95 per cent confidence intervals. TNM, tumour node metastasis. *Model 1: exploratory variables were sex, primary tumour, lymph node, distant metastasis, tumour grade, residual tumour and venous invasion (model $\chi^2 = 0{\cdot}0011$); model 2: exploratory variables were sex, TNM stage, tumour grade, residual tumour and venous invasion (model $\chi^2 = 0{\cdot}0028$)

Table 4 Correlation of microscopic invasion findings of the primary tumour with liver metastasis in patients who had curative (R_0) resection

	Liver metastasis		
Microscopic findings	Present	Absent	P*
Vascular invasion			
Present (n = 11)	7	4	0·033
Absent (n = 13)	2	11	
Lymphatic invasion			
Present (n = 16)	8	8	0·18
Absent (n = 8)	1	7	
Perineural invasion			
Present (n = 14)	5	9	> 1·0
Absent (n = 10)	4	6	

*Fisher's exact test

Table 5 Correlation of postresection tumour residue and stage grouping

		Residual tumour			
Stage	n	R_0	R_1	R_2	P*
I	8	8	0	0	< 0·0001
II	5	5	0	0	
III	8	5	3	0	
IVA	26	7	18	1	
IVB	10	1	4	5	
Total	57	26	25	6	

R_0, no residual tumour; R_1, microscopic residual tumour; R_2, macroscopic residual tumour. *χ^2 test

Superimposed on this finding, there was a significant ($P < 0·0001$) correlation between disease stage and curability of resection (*Table 5*). Rijken *et al.*[14], Kayahara *et al.*[10] and Takao *et al.*[2] reported higher R_0 resection rates than those found in the present study (46 per cent), of 53, 66 and 72 per cent respectively. Nevertheless, their 5-year survival rates were lower than the present ones (63 per cent for R_0, 16 per cent for R_1 and 0 per cent for R_2), not only after R_0 resection (40 per cent[14], 48 per cent[10] and 43 per cent[2]) but also after non-curative resection, 0 per cent[10,14] and 12 per cent[2]. There may be a misdirection of R_1 disease into R_0 disease in those reports presenting high curative resection rates, and this may also have occurred in the present report because of the six locoregional failures after R_0 resection. However, there is no doubt that tumour involvement of the surgical margin is an important matter of concern from the point of view of achieving curative resection.

The periductal soft tissue margins, or the radial ductal margin, had the highest risk of microscopic tumour residue because of the proximity to hepatic arteries and portal vein. It might be reasonable to attempt to resect these vessels together with tumour-bearing extrahepatic bile duct for patients with evident involvement. However, the problem will still occur where there are verified cancer cells in surgical margins despite macroscopic lack of involvement during operation. Also, as the scope of resection expands, such as with a complete hepatoduodenal ligamentectomy, a better curative resection rate might be obtained, but at the risk of higher morbidity and mortality rates[15].

Beneficial results from adjuvant radiotherapy in patients with advanced bile duct cancer were demonstrated, although this study was not a randomized trial. While the efficacy of adjuvant radiotherapy is controversial, previous studies on hilar bile duct cancer, gallbladder cancer and experimental tumours have shown statistically significant benefits from adjuvant radiotherapy in patients with microscopic residual disease[16–18]. In contrast, there are reports suggesting that radiotherapy does not improve the survival of patients with distal cholangiocarcinoma[1,19] or hilar bile duct cancer[20], although these studies did not stratify patients by R status in order to determine the effect of radiation therapy on patients with R_1 disease.

Ideally, a prospective randomized trial should be undertaken to determine whether adjuvant radiotherapy in patients with R_1 resection is beneficial or not. Unfortunately, the present data demonstrated that the most frequent site of distant metastasis following R_0 resection was the liver, which is in agreement with the recent report by Takao *et al.*[2], but differs from the *AJCC Cancer Staging Manual*[4] (which cites the lung instead). It is noteworthy that MVI of the primary tumour was a significant predictor of liver metastasis after R_0 resection. This finding indicates that MVI would be an appropriate patient selection factor for determining the appropriateness of adjuvant treatment to prevent liver metastasis, although at the moment there is no effective chemotherapy regimen. Recent experimental studies using human biliary tract cell lines suggest that protracted low-dose 5-fluorouracil administration could enhance the benefit of radiation therapy and inhibit the growth of microscopic metastasis following R_1 resection[21,22].

In conclusion, for patients with middle and lower third bile duct cancer, R_0 resection is the most favourable treatment. However, when surgical margins reveal cancer cell invasion, adjuvant radiotherapy might improve survival and could be considered. Of patients who have successful R_0 resection, those with MVI in the primary tumour are more likely to develop liver metastases. Therefore, even for those who undergo R_0 resection of the tumour with MVI, more effective postoperative systemic treatment strategies need to be developed in order to prevent liver metastasis in this seemingly dormant tumour phase.

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Original article

Impact of laparoscopic surgery on experimental hepatic metastases

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Background: Metastatic disease to the liver is one of the major factors determining outcome after colonic resection with curative intention. The influence of laparoscopic surgery on metastatic disease in the liver is still largely unknown.
Methods: An intrasplenic tumour cell inoculation was performed in 30 WAG-Rij rats. After 7 days the rats were randomized into three operative groups: laparotomy ($n = 10$), laparoscopy with 7 mmHg carbon dioxide pneumoperitoneum ($n = 10$) and gasless laparoscopy ($n = 10$). A small bowel segmental resection was carried out in all rats. Some 21 days later the rats were evaluated for number and diameter of tumour nodules and cancer index score at eight different abdominal sites.
Results: Hepatic tumour growth scored with the cancer index was significantly reduced in the gasless laparoscopy group compared with that in the carbon dioxide laparoscopy group ($P = 0{\cdot}04$) and the laparotomy group ($P = 0{\cdot}02$). Tumour growth at the port site and total tumour load were significantly reduced in the gasless group compared with the laparotomy group ($P \leq 0{\cdot}04$).
Conclusion: Laparoscopy with carbon dioxide insufflation seems to stimulate the growth of dormant tumour cells into overt liver metastases. Gasless laparoscopy on the other hand may have a protective effect against metastatic disease in the liver. The promoting and inhibiting effects of laparoscopic procedures on growth of liver metastases need further evaluation.

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Introduction

Laparoscopic colorectal surgery for malignancy has been claimed to be technically feasible and safe[1–3], but data are available on short-term outcome only. Enthusiasm has been limited by an increasing number of reports of port-site metastases and local tumour recurrence after laparoscopic cholecystectomy for unsuspected gallbladder carcinoma[4] and laparoscopic colectomy for colonic cancer[5,6]. Numerous investigations have followed, but the true incidence of port-site metastases and tumour recurrence after laparoscopy in comparison to conventional procedures is still under debate[7–10].

What has not been taken into account yet is the influence of laparoscopic procedures on the development of hepatic metastases. Since metastatic disease is one of the major factors determining outcome after colonic resection with curative intent, the influence of laparoscopy on this process is a matter of utmost importance. More than 50 per cent of patients with primary colorectal cancer develop hepatic metastases eventually[11]. It is assumed that micrometastases exist in the liver of these patients in a dormant state[11]. Factors that cause these dormant cells to develop into overt metastases are still unknown. In all probability unimpaired function of both the immune system and the liver has great influence on hepatic resistance to tumour cells. Laparoscopy preserves general immune function better than laparotomy[12–14]. On the other hand, previous studies have shown that increased intra-abdominal pressure due to the pneumoperitoneum is responsible for a number of adverse local side-effects, which may promote the development of hepatic metastases. Gasless laparoscopy, which does not produce these adverse local effects, might possess advantages over carbon dioxide pneumoperitoneum with regard to metastatic disease.

The purpose of this study was to investigate the influence of different surgical procedures on growth of hepatic metastases. A standardized technique of laparoscopic surgery in the rat[15] and a liver metastasis model with intraportal injection of tumour cells[16] have been described previously. In the present study this technique was modified to include intrasplenic injection of tumour cells 7 days before surgery, which may result in a more realistic flow of tumour cells into the portal system and in the presence of dormant cells in the liver at the time of surgical manipulation.

Materials and methods

Animals

Thirty male rats of the inbred WAG/Rij strain weighing 150–200 g were kept under standard laboratory conditions. All experimental procedures were approved by the National Research Council and complied with the national legislation governing the care and use of laboratory animals.

Tumour

CC531 is a 1,2-dimethylhydrazine-induced, weakly immunogenic, moderately differentiated colonic adenocarcinoma, transplantable in syngeneic WAG/Rij rats. The cells were cultured in RPMI 1640 medium supplemented with 2 per cent Hepes buffer solution, 1 per cent L-glutamine, 1 per cent streptomycin penicillin and 5 per cent fetal calf serum at 37°C in an atmosphere of 5 per cent carbon dioxide. Before use, cells were washed with phosphate-buffered saline, trypsinized for 5 min, centrifuged for 5 min at 1200 r.p.m., resuspended and counted. Viability was measured using the trypan blue method and always exceeded 95 per cent. To establish the tumour model, a concentration of 50 000 cells/ml was used.

Liver metastasis model

In all rats intrasplenic tumour cell inoculation was performed during laparoscopy with carbon dioxide pneumoperitoneum. A Veress needle was inserted into the abdominal midline and carbon dioxide pneumoperitoneum with a pressure of 7 mmHg was created using an electronic laparoflator (Aesculap, Tuttlingen, Germany). A laparoscopic sheath with an insufflation side port was inserted mid-abdominally, followed by a 4-mm arthroscope. In the right flank of the lower abdomen, a 5-mm incision was made and a blunt endoscopic 3-mm forceps was inserted into the abdominal cavity. The spleen was grasped and fixed with the forceps. A 0·4-mm cannula was inserted percutaneously into the abdominal cavity to puncture the spleen under direct vision. Some 0·1 ml of the tumour cell suspension (50 000 cells/ml) was injected. Seven days after intrasplenic tumour cell injection small bowel resection was carried out with different operative methods.

Operative groups

The animals were randomized into three different operative groups: a conventional laparotomy group ($n=10$), a laparoscopy group with carbon dioxide pneumoperitoneum ($n=10$) and a gasless laparoscopy group ($n=10$). All procedures were performed by one surgeon with wide experience of the different surgical techniques.

Operative procedures

The rats were anaesthetized by intraperitoneal injection of ketamine 100 mg/kg and Xylatinhydrochlorid 2 per cent 10 mg/kg.

For conventional laparotomy, the animals underwent a 5-cm midline incision. After 45 min, mobilization of the small bowel was carried out and a 1-cm segment was resected. An end-to-end anastomosis was performed using a 7/0 suture. Retractors were left *in situ* for 90 min. Then the abdomen was closed in one layer with a running suture.

For laparoscopy with carbon dioxide pneumoperitoneum, a Veress needle was inserted into the midline of the abdomen, two-thirds of the way between the xiphoid process and pubis. Carbon dioxide pneumoperitoneum of 7 mmHg was created as described. A laparoscopic sheath with an insufflation side port was inserted mid-abdominally, followed by a 4-mm arthroscope. Under direct vision, two operating ports were implanted in the lower abdomen. Forty-five minutes later a 1-cm mini-incision was made, a small bowel segment was extracted through this incision and extracorporeal resection of 1 cm in length was performed. End-to-end anastomosis was also performed extracorporeally using a 7/0 suture. After a total operating time of 90 min, the trocars and arthroscope were removed, and incisions were closed with a single suture in one layer.

In the gasless laparoscopy group an abdominal wall lift was performed mechanically using two abdominal retraction wires. At first the optic trocar was inserted into the abdominal cavity. Retraction wires could now be placed in both flanks under direct vision. With flexible arms installed on the small animal operating table, tension was brought on the retraction wires. Next a laparoscopic sheath and 4-mm arthroscope were inserted and two operating ports were implanted. Minilaparotomy, resection of a small bowel segment and reanastomosis were performed as described for the pneumoperitoneum group 45 min after set-up. Total operating time was 90 min. Trocars were removed and incisions closed with one suture in a single layer.

Postoperative studies

Twenty-one days after surgery all animals were killed and intraperitoneal tumour growth was scored by diameter and number of tumour nodules, and by a semiquantitative cancer index (*Table 1*). The index was assessed by two independent observers. In case of disagreement between the two observers the lower score was accepted. Tumour growth was evaluated in the subcutis (abdominal wall metastases), parietal peritoneum, mesentery, liver, kidney, retroperitoneum, omentum and scrotal fat. For assessment, organs were not removed from the abdominal cavity and only superficial lesions were taken into account. A random sample of tumour nodules was examined histologically to confirm metastatic growth.

Statistical analysis

Sample size was calculated following evaluation of preliminary studies. Mean(s.d.) values were calculated. To analyse the data for significant differences between the groups, the Kruskal–Wallis test (global test) and the Dunn test with α correction were performed.

Results

All animals survived anaesthesia and operative procedures, and could be evaluated 21 days after surgery. Disagreement between the two independent assessors occurred twice. No unexpected death or morbidity occurred after resection of a small bowel segment. Tumour growth was found in all rats to a different extent.

Splenic tumour growth

In none of the 30 rats was splenic tumour growth detected.

Hepatic tumour growth

Hepatic metastases developed in all rats in both the carbon dioxide and the laparotomy group but in only eight of ten rats in the gasless group (*Table 2*). The cancer index in the gasless laparoscopy group was significantly less than that in the carbon dioxide group ($P = 0{\cdot}04$) or in the laparotomy group ($P = 0{\cdot}02$) (*Fig. 1*). There was no significant difference between the carbon dioxide and laparotomy groups. The diameter of hepatic tumour nodules ranged from 1 to 4 mm in the carbon dioxide group, from zero to 3 mm in the gasless group and from 1 to 3 mm in the laparotomy group (P not significant). The number of hepatic tumour nodules varied between five and 70 in the carbon dioxide group, between zero and 27 in the gasless group, and between four and 48 in the laparotomy group (P not significant).

Table 1 Cancer index scoring

Tumour index	Size of tumour nodules
0	No tumour present
1	Diameter less than 0·5 cm
2	Diameter between 0·5 and 1·0 cm
3	Diameter between 1·0 and 2·0 cm
4	Diameter between 2·0 and 3·0 cm
5	Diameter exceeding 3·0 cm

Table 2 Proportion of rats affected by hepatic and port-site metastases, and total tumour load in three experimental groups

	Hepatic metastases	Port-site metastases	Total tumour load
Carbon dioxide laparoscopy	10 of 10	8 of 10	10 of 10
Gasless laparoscopy	8 of 10	3 of 10	10 of 10
Laparotomy	10 of 10	9 of 10	10 of 10

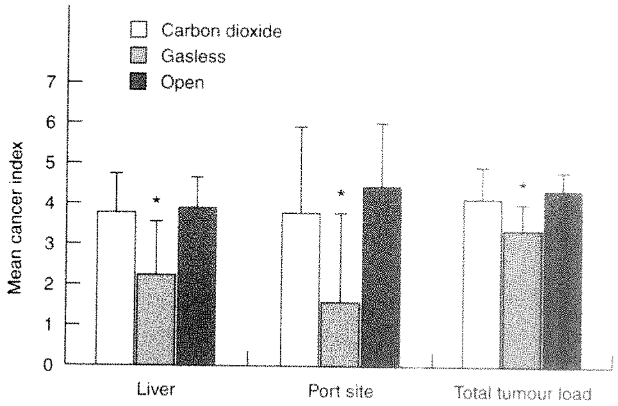

Fig. 1 Mean(s.d.) cancer index of the three operative groups at the liver and port site, and total tumour load (mean cancer index of all evaluated abdominal sites). $^*P < 0{\cdot}05$ *versus* carbon dioxide and open group (Kruskal-Wallis test and Dunn test with α correction)

Port-site tumour growth or wound recurrence

Port-site tumour growth in the laparoscopy groups and wound recurrence in the laparotomy group was located in the subcutis. Port-site metastases were found in eight of ten animals in the carbon dioxide group and in three of ten animals in the gasless group. Wound recurrence was found in nine of ten animals in the laparotomy group. The cancer index was significantly lower in the gasless laparoscopy group than in the laparotomy group ($P = 0{\cdot}04$); no other significant differences were found. The diameter of port-site metastases ranged from zero to 15 mm in the carbon dioxide group and from zero to 6 mm in the gasless group. The diameter of wound recurrence ranged from zero to 5 mm in the laparotomy group. Differences failed to show any significance. The number of tumour nodules at the port site or wound, respectively, was significantly decreased in the gasless group compared with the laparotomy group ($P = 0{\cdot}001$).

Total tumour load

Tumour growth was assessed collectively at the aforementioned abdominal sites. The cancer index in the gasless laparoscopy group was significantly lower than that in the laparotomy group ($P = 0{\cdot}03$). There were no significant differences between the other experimental groups. The number and diameter of tumour nodules in the three experimental groups were not significantly different.

Discussion

The true incidence of local tumour recurrence and port-site metastases after laparoscopic surgery is still unclear[7,10]. Apart from this, in terms of colorectal surgery for carcinoma, one important question to be answered is whether laparoscopy with or without gas insufflation influences the process of metastatic disease in the liver. Liver metastases are present in some 25 per cent of patients at the time of initial colorectal resection and more than 50 per cent of patients with colorectal carcinoma will eventually develop them[11]. There is much evidence for the theory that micrometastases already exist in a dormant state in the liver of patients with colorectal cancer. At the time of primary surgery it is important not only to avoid additional tumour cell detachment from the primary tumour and subsequent migration into the portal venous circulation but also to prevent any adverse events in the liver, which could promote dormant malignant cells to grow into overt metastasis. It is therefore important to know which changes in liver function might cause the dormant cells to develop into an aggressive form with malignant behaviour. It is assumed that unimpaired immune and liver function contribute to a considerable degree to hepatic tumour cell resistance. In this respect, changes in hepatic haemodynamics also appear to be of major importance.

In the present study a liver metastasis model in the rat was used to investigate the influence of different surgical procedures on the development of overt metastases in the liver using an intrasplenic injection of tumour cells 7 days before small bowel resection. Several models of experimental liver metastasis are mentioned in the literature. Direct intraportal injection[17], intracaecal[18], subcutaneous[18] and intrasplenic[19] injection have been described so far. In this study intrasplenic injection was chosen, because this model guarantees transportation of tumour cells to the portal system and provides a continuous flow of cells to the liver. This flow of cells should imitate the real situation of blood drainage into the portal system better than a bolus injection of cells directly into the portal vein. Furthermore, induction of hepatic metastasis is more reliable after intrasplenic tumour cell injection than after distant intracaecal and subcutaneous injection.

The cancer index for the liver was significantly lower in the gasless laparoscopy group than in the carbon dioxide and laparotomy groups. A significantly smaller cancer index and number of tumour nodules were found at the port site in the gasless laparoscopy group compared with the laparotomy group. Furthermore, the total tumour load in the gasless laparoscopy group was significantly smaller than that in the laparotomy group. These results are in accordance with those of a previous studies[16], in which an intraportal tumour cell inoculation was performed to induce hepatic tumour spread. In the latter study the hepatic tumour growth and total tumour load were significantly smaller in the gasless laparoscopy group than in the carbon dioxide pneumoperitoneum and laparotomy groups.

The laparoscopic approach has been shown to preserve general immune function better than laparotomy[12,13]. The lesser impact on immune function could be the main advantage of gasless laparoscopy in respect of metastatic disease. Laparoscopy with carbon dioxide pneumoperitoneum also preserves immune function, but the results of the present study suggest that pneumoperitoneum must also promote hepatic metastasis.

Previous studies support the hypothesis that carbon dioxide pneumoperitoneum results in changes to liver function which enable dormant tumour cells to grow into overt liver metastases. Although general immune function seems to be preserved by using minimally invasive procedures, laparoscopy with pneumoperitoneum in the rat led to a significant reduction in the activity of the mononuclear phagocytic system in comparison to laparotomy and gasless laparoscopy[21]. In a study of intraperitoneal haemodynamics, Kotzampassi *et al.*[20] found a raised portal pressure in the carbon dioxide group as a result of compression of this large vessel. Jakimowicz *et al.*[22] demonstrated a significant decrease in portal flow with increasing intraperitoneal pressure in patients undergoing laparoscopic cholecystectomy. These results were also confirmed in a rat model[23]. Eleftheriadis *et al.*[24] demonstrated a significantly decreased hepatic microcirculation in patients undergoing laparoscopic surgery in relation to controls. In 52 patients treated with different laparoscopic procedures Morino *et al.*[25] found that the duration and level of intra-abdominal pressure were responsible for increased levels of aspartate aminotransferase, alanine aminotransferase and bilirubin, and prolongation of prothrombin time.

In conclusion, the systemic advantage conferred by laparoscopy seems to be compromised by the local effects of carbon dioxide pneumoperitoneum on the liver. Only by using gasless laparoscopy does the advantage of better immune function result in significantly reduced growth of metastases in the liver. However, this cannot yet be extrapolated to humans because gasless laparoscopic colonic resection is still beset by technical difficulties.

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Original article

Clinicopathological features and treatment of intraductal papillary mucinous tumour of the pancreas

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Background: The surgical strategy in patients with a pancreatic intraductal papillary mucinous tumour (IPMT) is still controversial. In this study the pathological findings in a series of patients were used to rationalize surgical choice.

Methods: Fifty-one patients with IPMT were observed between 1988 and 1998 and treated by pancreatic resection. Factors evaluated included symptoms, tumour site, type of operation, histological findings and resection margins, tumour stage, follow-up and survival.

Results: Pancreaticoduodenectomy was the most frequent surgical treatment (33 patients; 65 per cent), followed by left pancreatectomy (ten), total pancreatectomy (five) and middle pancreatectomy (three). Histological assessment revealed the tumour to be an adenoma in 13 patients (25 per cent), a borderline tumour in ten (20 per cent) and a carcinoma in 28 (55 per cent), 19 of which were invasive. Mild to moderate dysplasia was present at the resection margin in 20 specimens (41 per cent), and carcinoma in one. Local recurrence was observed in four patients (8 per cent), all of whom underwent a second resection. The 3-year actuarial survival rate for benign and malignant disease was 94 and 69 per cent respectively ($P = 0.03$).

Conclusion: These results suggest that resection should be the treatment for IPMT. Management of the resection margin could be crucial in avoiding tumour recurrence.

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Introduction

The term intraductal papillary mucinous tumour (IPMT) denotes a new pancreatic lesion introduced recently in the World Health Organization (WHO) classification[1–3], presenting different pathological patterns: intraductal growth, diffuse or segmental dilatation of the main and/or secondary pancreatic ducts, and absence of neoplastic stenosis. Histologically, the tumour includes a spectrum of changes ranging from low- to high-grade dysplasia and from adenoma to infiltrating carcinoma[1–7]. A precise classification has now enabled a review of histological preparations[8,9] and rejection of alternative names[2,10].

There has been increasing interest in these tumours, their incidence[4,8,11,12], their diagnostic recognition[13–15], their prognosis compared with ductal carcinoma[2,9,10,16], and the ability to identify clinical or morphological signs, allowing preoperative diagnosis[2,4,8,10,11,17].

The lesion mainly affects the head of the pancreas and the uncinate process[4,8,9,17,18], and tends to progress to carcinoma[1,15], although the timescale is unknown[2,6,19,20]. These data constitute the indication for resection of the part of the gland most affected[2,8,17] and for intraoperative monitoring of the pancreatic resection margin[2,8,9,17,18]. There remain problems when recommending surgery to an elderly patient[2,9,17] for a tumour that may still be benign. Second, there is the difficulty of intraoperative histological examination of the resection margin, which rarely furnishes an unambiguous 'carcinoma negative' response[8,9,21].

The aim of this study was to use pathological findings to clarify the surgical strategy that should be adopted for patients with IPMT.

Patients and methods

Between 1988 and 1998, 63 patients with IPMT were treated; 51 had a surgical resection and constitute the study group for analysis. There were 27 men and 24 women with a mean age at diagnosis of 64 (range 34–76) years. All patients had abdominal ultrasonography and/or computed tomography (CT). In 31 patients (61 per cent) the tumour was located in the head of the pancreas and/or in the uncinate process, in seven it involved the head and body, in three the body alone and in ten the body and tail of the pancreas. Four patients had undergone pancreatic anastomotic surgery 37, 40, 78 and 115 months before pancreatic resection. Pancreatic surgery involved standard surgical resections except in three patients who had a middle pancreatectomy, in which the pancreatic body was resected, burying the head stump and constructing a pancreaticojejunal anastomosis at the tail. All patients underwent standard lymphadenectomy.

Clinical data regarding all pancreatic tumours were collected prospectively and entered into a computer database. At follow-up, patients had abdominal ultrasonography or CT; serum tumour markers were estimated 6 months after operation and repeated at yearly intervals.

Table 1 Clinical details of 51 patients with intraductal papillary mucinous tumour in relation to tumour subtype

	IPMA/B ($n=23$)	IPMC-NI/C-I ($n=28$)
Sex ratio (M : F)	14 : 9	13 : 15
Mean (range) age (years)	59·4 (38–76)	63·2 (34–76)
Alcohol > 40 g/day	6	7
Smoker	12	12
Coffee drinker	21	23
Symptoms*		
Recurrent pain	19	15†
Weight loss	8	14
Onset or deterioration of diabetes	1	9‡
Jaundice	0	5
None	3	4
Previous acute pancreatitis	6	6
Previous chronic pancreatitis	4	2
Previous pseudocyst	4	2
Previous pancreatic surgery	2	2

IPMA/B, intraductal papillary mucinous adenoma and borderline tumours; IPMC-NI/C-I, intraductal papillary mucinous carcinoma – non-invasive, and carcinoma – invasive. *Some patients had more than one symptom. †$P = 0{\cdot}04$, ‡$P = 0{\cdot}01$ *versus* IPMA/B (Fisher's exact test)

Pathology

The first prospective pathological diagnosis was made in 1992, and in a further five patients IPMT was diagnosed on the basis of a review of histological preparations. Resected specimens were examined fresh and unfixed, and the bile and main pancreatic ducts were probed. The whole specimen was cut horizontally along the probes and fixed immediately in 10 per cent formalin, as described previously[22]. Four to five successive, numbered specimens were sectioned and a mean of 14 (range 8–20) blocks were examined. In 16 patients, an additional specimen containing a cross-section of the entire lesion was examined. The surgeon and pathologist evaluated the specimens first by obtaining a frozen section of the pancreatic resection margin and then by removing local lymph nodes. At intraoperative examination the pancreatic resection margin was considered negative (normal ductal epithelium, simple mucinous metaplasia) or positive in the presence of unmistakable high-grade dysplastic alteration of the ductal epithelium. All tissues were embedded in paraffin, sectioned and stained with haematoxylin and eosin, and with Alcian blue (1 per cent; pH 2·5) and periodic acid–Schiff.

All specimens were examined histologically and the tumours were typed and graded as adenomas (IPMA), borderline tumours (IPMB) and carcinomas (non-invasive (IPMC-NI) and invasive (IPMC-I)) according to the WHO[1] and Armed Forces Institute of Pathology[3] classifications. Adenoma was characterized by mild epithelial dysplasia, borderline tumour by moderate dysplasia, and carcinoma by high-grade dysplasia or carcinoma *in situ*. The invasive component was considered muconodular (IPMCM), characterized by the presence of mucin-filled cystic spaces partly lined with atypical cells and containing floating mucus-secreting cells, or tubular (IPMCD), resembling the usual ductal adenocarcinoma[23].

Statistical analysis

Non-parametric tests (Kruskal–Wallis, Mann–Whitney *U* and Spearman correlation tests) were used to evaluate ordinal data, and the χ^2 test was used for analysis of nominal data. In the two-way contingency tables, Yates' continuity correction was applied, or Fisher's exact test in the case of small expected frequencies. The actuarial analysis was conducted by means of Kaplan–Meier survival curves with the Mantel–Cox test for group comparisons; the endpoint considered was death.

SPSS® release 8.5 (SPSS, Chicago, Illinois, USA) statistical programs were used.

Results

There were no statistically significant differences in clinical parameters between patients with benign or malignant IPMT. Exceptions were the incidence of recurrent episodes of pain, which were more common in patients with a benign tumour, and the number of patients with recent onset or deterioration of diabetes, which was more common in malignant disease (*Table 1*). *Table 2* summarizes the surgical procedures performed in relation to the definitive histological findings on the surgical specimens.

Table 2 Tumour subtype according to the World Health Organization classification[1], in relation to the operation performed in 51 patients with intraductal papillary mucinous tumours

	Tumour subtype				
Operation	Total	IPMA	IPMB	IPMC-NI	IPMC-I
Whipple resection	15	2	4	1	8
Extended Whipple resection	2	0	0	0	2
PPPD	15	7	2	3	3
Extended PPPD	1	0	0	0	1
Splenopancreatectomy	10	3	3	3	1
Middle pancreatectomy	3	0	1	1	1
Total pancreatectomy	5	1	0	1	3
Total	51	13	10	9	19

IPMA, intraductal papillary mucinous adenoma; IPMB, intraductal papillary mucinous borderline tumour; IPMC-NI, intraductal papillary mucinous carcinoma – non-invasive; IPMC-I, intraductal papillary mucinous carcinoma – invasive; PPPD, pylorus-preserving pancreaticoduodenectomy

The postoperative course after the initial operation was complicated in 25 patients (49 per cent). One patient (2 per cent) died. Nineteen patients had abdominal complications, including 14 with a pancreatic fistula, defined as an output greater than 10 ml per day from postoperative day 4, and with an amylase content three times greater than the serum value. The mean(s.d.) duration of postoperative hospital stay was 20(12) days and was significantly shorter in patients with an uneventful postoperative course (13(3) *versus* 28(13) days; $P < 0{\cdot}001$).

Pathology

Macrosopically, two main types of IPMT were distinguished: 22 were of the 'ductectatic mucin-hypersecreting' type in which the dilated ducts were filled with tenacious mucin and histologically the epithelial lining was flat or presented tiny microscopic papillae. The other 29 were of the 'papillary villous' type, characterized by polypoid papillary proliferations associated with mucous material within the dilated pancreatic duct (*Table 3*). The growth pattern correlated with tumour subtype (*Table 3*): the adenomas had a ductectatic pattern, whereas the infiltrating carcinomas presented a papillary pattern. Of the 19 patients with infiltrating lesions, perineural and microvascular infiltration was present in seven and four respectively. Lymph node metastasis was detected in ten patients; all had an infiltrating tumour.

In all, 49 resection margins were examined. Carcinoma was present in the resection margin from one patient with the diffuse intraductal form of IPMT. *Table 4* summarizes the histological findings at the resection margins in relation to tumour subtype. Definitive examination of the resection margins confirmed the diagnosis obtained with the frozen sections. All of the four patients who presented with recurrent disease underwent a second resection; the resection margins from the first operation were de-epithelialized at the site of subsequent recurrence.

Table 3 Tumour growth pattern in 51 patients with intraductal papillary mucinous tumours

		Histological pattern	
Tumour subtype	*n*	Ductectatic	Papillary
IPMA	13	13	0
IPMB	10	5	5
Malignant tumours	28	4	24
IPMC-NI	9	4	5
IPMC-I	19	0	19
Muconodular	8	0	8
Ductal	11	0	11
Total	51	22	29

IPMA, intraductal papillary mucinous adenoma; IPMB, intraductal papillary mucinous borderline tumour; IPMC-NI, intraductal papillary mucinous carcinoma – non-invasive; IPMC-I, intraductal papillary mucinous carcinoma – invasive

Follow-up

The mean follow-up of the 51 patients was 15 (range 4–127) months. Four patients (8 per cent) developed local recurrence in the pancreas and underwent a second resection 37, 45, 58 and 62 months after the first. The recurrence affected the stump of the head in three of these patients who had undergone middle pancreatectomy and in a fourth who had undergone splenopancreatectomy. In all four, the second operation consisted of pancreaticoduodenectomy. The 3-year actuarial survival rate for benign and malignant tumours was 94 and 69 per cent respectively ($P = 0{\cdot}03$). In addition to the patient who died after operation, a further eight patients died during follow-up. One patient with an adenoma died from trauma; the other seven all had an infiltrating carcinoma and died from disease progression. In five of these patients the lymph nodes contained metastases. Their mean postoperative survival was 10·3 months; one patient is alive with advanced disease.

Discussion

In the presence of an IPMT the surgeon is faced with the problem of whether surgery is indicated or not. It is hard to

Table 4 Resection margins in 49* intraductal papillary mucinous tumours in relation to tumour subtype

Tumour subtype	Total	Resection margin Normal epithelium	Mild dysplasia	Moderate dysplasia	Severe dysplasia or carcinoma	De-epithelialized
IPMA	12	9	3	0	0	0
IPMB	10	7	2	1	0	0
IPMC-NI	8	3	2	3	0	0
IPMC-I	19	4	2	7	1†	5
Total	49	23 (47)	9 (18)	11 (22)	1 (2)	5 (10)

Values in parentheses are percentages. IPMA, intraductal papillary mucinous adenoma; IPMB, intraductal papillary mucinous borderline tumour; IPMC-NI, intraductal papillary mucinous carcinoma – non-invasive; IPMC-I, intraductal papillary mucinous carcinoma – invasive. *Includes two specimens from three patients who had intermediate resection, and five patients from whom no specimen was obtained, making a total of 49 specimens. †Patient refused total pancreatectomy

establish the benign or malignant nature of the lesion before operation[17]. A number of investigators have proposed anamnestic[8,23], laboratory, clinical and morphological[5,9,17] criteria associated with malignancy. In the present series, however, a number of these parameters including age, alcohol intake, cigarette smoking and duration of symptoms did not correlate with malignancy. Serum[5] or intracystic tumour markers do not help. Diagnostic techniques, such as ultrasonographically or CT-guided needle aspiration, brushing during endoscopic retrograde cholangiopancreatography (ERCP)[5,24], or the preoperative[7] or intraoperative[25] use of intrapancreatic probes, are unable to guarantee a complete map of the state of the epithelium. IPMTs may display all the various grades of dysplasia within a single lesion[4–7]; however, they are often malignant in a proportion that may be as low as 5 per cent or as high as 88 per cent[8,9,16,17,21,26–29].

One factor in favour of surgery is the fact that the better prognosis of these tumours compared with ductal adenocarcinoma is related to those lesions that are still premalignant[6,8,9,11]. In agreement with Azar *et al.*[21] and Cuillerier *et al.*[8], the survival rate was very poor when the disease had already reached the stage of lymph node involvement. This latter consideration necessarily biases surgeons towards extended lymphadenectomy[17], which to date has been regarded as superfluous[2,30,31]. An additional element in favour of surgery is the need to relieve the pain, which is a very common symptom[2,9,11]. It is often present in patients with the disease still in its initial stages (19 of 23 patients in the present series). Although repeated ERCP has yielded evidence of ductal clearance in certain patients[5,21], subsequent follow-up has shown the occurrence of neoplastic degeneration[5,21]. The risk of submitting this benign lesion to resection may still be justified if the perioperative morbidity and mortality rates are low[32]. In the light of all these considerations, the present authors agree with others[2,8,9,17,18,29] that resection is indicated in all fit patients with IPMT.

The oncologically correct approach to treatment should be to proceed with a pancreatic resection until a resection margin free of epithelial atypia is achieved[2,17], up to total pancreatectomy if necessary. In the literature, the need for total pancreatectomy ranges from 0 to 35 per cent[2,5,8,9,17,18,21]. Where it proves impossible to obtain a negative resection margin, many surgeons[8,9,21] stop resecting in the presence of mild or moderate dysplasia.

In the present study as many as 20 patients had mild to moderate epithelial dysplasia at the resection margin. Follow-up monitoring[5,31] is needed to detect recurrence, which may be expected in 0–52 per cent[2,8,9,16,17,21,31]. In this study recourse to an atypical resection was associated with a higher recurrence rate[18,31]. Three tumours apparently localized to the body of the pancreas were treated by middle pancreatectomy; all recurred at the cephalic resection margin. No recurrences were found at the resection margin after the 33 resections of the head of the pancreas, suggesting that in tumours located at the head–body border it is more useful to start the resection at the head and then to proceed caudally. The factor most clearly associated with recurrence, however, was the presence of de-epithelialization of the resection margin. This was wrongly interpreted as an absence of neoplasia. In four of these patients there was local recurrence of the tumour, and a fifth patient, who underwent pylorus-preserving pancreaticoduodenectomy, has had only a short follow-up. The presence of de-epithelialization should, therefore, be regarded as a 'positive resection margin'.

The results of this study confirm that resection is the treatment of choice for IPMT. Management of the resection margin could be of crucial importance. Firm evidence of malignancy should prompt a more radical resection, including total pancreatectomy, if necessary. The extent of the resection in patients with mild to moderate dysplasia at the resection margin remains uncertain, pending the results of studies with a longer follow-up.

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Original article

Epidemiology of abdominal aortic aneurysms in the Asian community

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Background: Studies relating to the ethnic origin of patients with an abdominal aortic aneurysm (AAA) are few and are mainly concerned with the differences between black and white Americans. The purpose of this study was to determine whether the incidence of AAA among the Asian population of Bradford is different from that in the Caucasian population.

Methods: A retrospective study of patients with an AAA was carried out between 1990 and 1997 using data collected by the Patient Administrative Service, personal databases of the vascular consultants and theatre records. Information about the ethnic composition of the population of Bradford was obtained from the 1991 national census. Demographic data, including ethnic origin and clinical details, were obtained from patient notes.

Results: Two hundred and thirty-three patients with an AAA were identified during the study interval. The Asian population comprised 14·0 per cent of the total population of Bradford. Twenty-eight AAAs would be expected per year. All of the aneurysms identified occurred in the Caucasian population and none in the Asian community.

Conclusion: These early results suggest that AAA is rare among the Asian population.

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Introduction

Abdominal aortic aneurysm (AAA) is the tenth leading cause of death in older men[1]. The incidence is reported as 11·3 per 100 000 in men and 3·0 per 100 000 in women[2]. Fundamental issues about the cause and epidemiology of the condition remain unresolved.

The traditional view is that AAAs are a manifestation of atherosclerosis. This is based on the common risk factors and the coexistence of atherosclerosis and aneurysm disease. It is supported by evidence from resected specimens which show histological features of atherosclerosis and the propensity of the infrarenal aorta, which is prone to atherosclerosis, to become aneurysmal[3,4].

This view has been challenged by recent studies that suggest a role for specific genetic factors. Tilson[5] has suggested that atherosclerosis is a secondary phenomenon, proposing that risk factors such as hypertension, smoking and hypercholesterolaemia either cause aneurysmal or occlusive disease, depending on an individual's genetic predisposition.

If there is a genetic influence it is likely that different ethnic groups will have a different incidence of AAA. Research into this is scarce. La Morte *et al.*[6] found that white patients were more likely to have an AAA, while black patients had more vascular risk factors and underwent femoral artery bypass surgery more often. Interestingly, the Eastern Asian community has been shown to have a progressive increase in AAA disease over the past 21 years, similar in time frame and pattern to that reported in the West[7].

The aim of the study was to establish the incidence of AAA in the population of Bradford and to determine if there is a difference in incidence between the Asian and Caucasian populations.

Methods

Data were gathered retrospectively for the years 1990–1997. Patients with an AAA were identified from the Patient Administration Service and theatre records. Ethnic origin was established from coding (International Classification of

Diseases, 9th Revision, Clinical Modification), surname and religion using the patient notes. Population statistics were obtained from the Bradford City Council 1991 census records.

Results

The total population of Bradford is 475 437. According to the Bradford City Council census records 14·0 per cent of the population is Asian (*Table 1*). Health Authority data, however, have suggested this to be an underestimate and the figure may be as high as 30 per cent.

Some 23·3 per cent of the white male population of 193 131 was over the age of 55 years. Only 16·3 per cent of the total male Asian population of 33 894 was aged over 55 years, giving a population of 5525 at risk (*Table 2*). Some 30·6 per cent of the female white population was over 55 years old. Only 14·2 per cent of the 31 575 female Asians were aged over 55 years, giving a group of 4484 at risk.

Two hundred and thirty-three patients with an AAA were identified for the interval studied. The male to female ratio was 8 : 1 and the age range was 48–92 years. Using the figures quoted by Fowkes *et al.*[2] the expected incidence was 28 cases per year (actual 33 per year). In the Asian at-risk population of 10 009 no cases of AAA were identified, compared with 233 in the white population at risk of 107 862 ($P < 0{\cdot}001$, 1 d.f., χ^2 test).

Table 1 Population of Bradford by ethnic origin (1991)

	n
White	398 281 (83·6)
Asian	65 469 (14·0)
Other	11 687 (2·4)

Values in parentheses are percentages

Table 2 Male population of Bradford by age and ethnic origin (1991)

	< 55 years	> 55 years	Total
White	148 073 (76·7)	45 058 (23·3)	193 131
Asian	28 369 (83·7)	5525 (16·3)	33 894
Other	5633 (91·3)	540 (8·7)	6173
Total	182 075 (78·1)	51 123 (21·9)	233 198

Values in parentheses are percentages

Table 3 Female population of Bradford by age and ethnic origin (1991)

	< 55 years	> 55 years	Total
White	142 346 (69·4)	62 804 (30·6)	205 150
Asian	27 091 (85·8)	4484 (14·2)	31 575
Other	5116 (92·8)	398 (7·2)	5514
Total	174 553 (72·1)	67 686 (27·9)	242 239

Values in parentheses are percentages

Discussion

The study may be criticized because a certain amount of miscoding of procedures may have taken place. Misclassification of age, sex and even race may have occurred, but only in a relatively small percentage of cases and it was unlikely to have had a major impact.

The at-risk Asian population was also relatively small (10 009 patients) compared with the at-risk white population of 107 862. This may also be inaccurate; the Health Authority records differ from the census data in that the Asian population may represent 30 per cent of the total population of Bradford. Also the population has aged since the census, resulting in a greater at-risk population.

Differential access to medical care on the basis of race or socioeconomic status could have resulted in selection bias. Most AAAs are asymptomatic and are discovered during physical examination or abdominal imaging for other reasons. Therefore it is possible that persons with limited access to regular health care would be less likely to have an aneurysm diagnosed. However, data on vascular complications such as stroke and peripheral vascular disease show that Indo-Asians do utilize the health service[8].

Several conclusions can be drawn from this study. First the overall incidence of AAA in the white population of Bradford was similar to that expected. Second, AAAs were far more common in white than Asian men. Very little is published regarding the incidence of atherosclerotic disease in the Asian population, although it is reported to occur[9]. Black patients similarly have a high incidence of femoropopliteal occlusive disease but a low incidence of AAA[6,10]. Although both AAAs and femoral arterial occlusive disease have traditionally been thought of as manifestations of atherosclerosis, this study casts further doubt on the assumption that aneurysms occur purely as a consequence of atherosclerosis and adds support to the theory that they occur as a consequence of a genetic predisposition[11].

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Preliminary study of D-dimer as a possible marker of acute bowel ischaemia

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Background: Occlusion of the superior mesenteric artery (SMA) demands prompt recognition and diagnosis. No accurate diagnostic method is available. The aim of this study was to determine whether the fibrinolytic marker D-dimer is a useful early marker of acute bowel ischaemia.
Methods: Fourteen patients suspected of having acute bowel ischaemia were analysed for an increase in plasma D-dimer level.
Results: Six patients had embolic or thrombotic occlusion of the SMA and all had significantly higher D-dimer levels than those without thromboembolic occlusion ($P < 0.05$). Four patients with strangulation of the small bowel due to adhesions and one with a ruptured aortic aneurysm also had raised D-dimer values.
Conclusion: In patients with suspected thromboembolic occlusive disease of the SMA, a raised level of D-dimer indicated the presence of acute bowel ischaemia, whatever the cause. A more extensive prospective study is needed to evaluate a potential survival benefit using the test as a marker of the need for urgent laparotomy.

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Introduction

Prompt recognition, diagnosis and treatment are essential for successful outcome in the management of patients with acute bowel ischaemia. Occlusion of the superior mesenteric artery (SMA) must be considered when there is a sudden severe abdominal pain with initially minimal abdominal signs. Pain out of proportion, accompanied by rapid and often forceful bowel evacuation, and a possible source of embolus is the 'clinical triad' of acute bowel ischaemia.

Prognosis is poor; in a recent report from Glasgow[1], the mortality rate was 83 per cent. Neither clinical presentation, white blood cell count nor serum amylase measurement are helpful in the diagnosis. Immediate laparotomy or angiography with subsequent thrombolysis[2,3] are the treatment options available. A diagnostic method to select patients with acute abdominal pain in need of immediate intervention could improve survival rates. Laboratory signs such as plasma lactate concentration[4] and leucocytosis[5] have low specificities. Metabolic acidosis occurs as a consequence of severe intestinal ischaemia and necrosis, but develops late[6]. Contrast-enhanced computed tomography and plain radiography are not specific[7]. Duplex sonography can be useful in detecting proximal SMA occlusion[8] and is almost as accurate as angiography in the diagnosis of high-grade stenosis or occlusion of the SMA[9,10], but this method is very operator dependent.

The fibrinolytic marker D-dimer has been used extensively in the diagnosis of venous thromboembolism but the results remain controversial[11]. Several recent D-dimer assays have high sensitivity and negative predictive values, at least regarding proximal deep vein thrombosis, and especially pulmonary embolism. A chance finding of a raised D-dimer level on admission in a patient in this unit who had an embolus to the SMA, stimulated an interest. It was decided to perform a pilot study of D-dimer levels in patients with a clinical suspicion of acute bowel ischaemia, to determine whether a larger prospective study was worthwhile.

Patients and methods

Consecutive patients with a clinical suspicion of acute, occlusive mesenteric ischaemia due to thromboembolic disease of the SMA were studied. The patients were treated between October 1999 and July 2000 in Blekinge Hospital, Karlskrona ($n = 12$) or at the Skellefteå Hospital ($n = 2$). Blood samples were taken before operation, either on admission (nine patients), in the surgical ward (three) or in the intensive care unit (two) (*Tables 1* and *2*). D-dimer was analysed with a Nycocard® immunofiltration assay (Nycomed, Lidingö, Sweden) where the normal value is less than 0·3 mg/l.

Results

Six patients had verified thromboembolism to the SMA at laparotomy (*Table 1*) and eight had other conditions (*Table 2*). The mean age was 80 and 79 years respectively in the two groups. The mean(s.d.) preoperative D-dimer level was 4·7(3·3) mg/l in patients with SMA thromboembolism, higher than that in patients with other conditions ($P < 0·05$; unpaired t test).

The sensitivity, specificity and predictive values for a positive and a negative test were calculated for different outcomes. A patient with a ruptured abdominal aortic aneurysm (AAA) was considered to need a laparotomy, as well as four patients with bowel ischaemia from other causes. The sensitivity and predictive value of a negative D-dimer test was 100 per cent for SMA thromboembolism, for bowel ischaemia of any cause and for the need for laparotomy. The specificities were 38, 75 and 100 per cent respectively for these outcomes.

The finding of a raised D-dimer value pointed to the possibility of thromboembolic occlusion of the SMA in all five patients studied prospectively. In three this resulted in

Table 1 Clinical details of patients with thromboembolic occlusion of the superior mesenteric artery

Age (years)	Sex	AF/AMI	Clinical triad	D-dimer (mg/l)	Embolus/ thrombus	Revascularization	Bowel resection	Survival
63	F	No	No	6·9	Embolus	Embolectomy	Yes	3 days
92*	F	AF	Yes	2·4	Embolus	No	No	5 h
64	F	AF/AMI	Yes	1·3	Embolus	Embolectomy	Yes	37 days
84	M	No	No	3·2	Thrombus	Thrombectomy	Yes	Discharged
91	F	AF	Yes	4·1	Embolus	Embolectomy	Yes	6 days
88	M	AF	Yes	10·1	Embolus	No	Yes	2 days

*The patient was initially judged unfit for operation. D-dimer was analysed before operation (normal D-dimer range below 0·3 mg/l). Clinical triad is explained in the text. AF, atrial fibrillation; AMI, acute myocardial infarction

Table 2 Clinical details of patients with causes other than thromboembolic occlusion of the superior mesenteric artery

Age (years)	Sex	AF/AMI	Clinical triad	D-dimer (mg/l)	Diagnosis	Bowel resection	Outcome
84	F	No	No	0·4	Strangulation ileus with bowel ischaemia	Yes	Discharged
82	M	No	No	1·0	Ruptured abdominal aortic aneurysm	No	Discharged
90*	F	No	No	0·8 (2·0)	Strangulation ileus with bowel ischaemia	Yes	Discharged
57	F	No	No	2·3	Strangulation ileus with bowel ischaemia	Yes	Discharged
85	M	AF	Yes	< 0·3	Abdominal pain (not operated)	No	Discharged
78	M	Suspected AMI	Yes	< 0·3	Cholecystitis	No	Discharged
75	M	AF	Yes	< 0·3	Adhesions due to previous surgery	No	Discharged
77	F	AF	Yes	5·6	Strangulation ileus with bowel ischaemia	Yes	Discharged

D-dimer was analysed before operation (normal D-dimer range below 0·3 mg/l) except in one patient* in whom the measurement was repeated 2 days later, before laparotomy. Clinical triad is explained in the text. AF, atrial fibrillation; AMI, acute myocardial infarction early laparotomy and attempted revascularization. One survived after immediate laparotomy, thrombectomy of the SMA and bowel resection. Two patients had surgery too late to permit revascularization, despite an early suspicion of the condition. One patient was judged too ill for surgery and the other had already developed peritonitis due to intestinal gangrene when first evaluated.

Discussion

The fibrinolytic system is activated after arterial thromboembolic occlusion. In patients with acute ischaemic stroke[12] or leg ischaemia[13] levels of the fibrinolytic plasma markers thrombin–antithrombin III complex and D-dimer are raised. However, symptomatic arterial occlusion of the brain and of the leg seldom offer diagnostic difficulty, so fibrinolytic markers are of limited value. In contrast, mesenteric ischaemia remains a diagnostic challenge. Fibrinolytic markers may prove valuable, but they are not specific for vessel occlusion. Symptomatic AAA is associated with raised D-dimer levels[14]. Even patients with an asymptomatic AAA have significantly higher D-dimer levels before operation compared with values 19 months after surgery[15], suggesting an activated fibrinolytic system before surgery, probably due to the thrombus turnover within the aneurysm sac. Severe trauma[16] and surgery[17] also activate the fibrinolytic system, secondary to activation of the haemostatic system; this increase in D-dimer level remains for weeks[16,18]. Thus, the results of D-dimer tests cannot easily be interpreted in patients who have recently had surgery. The test manufacturer's stated normal range has been used as the cut-off level to diagnose deep vein thrombosis; whether this level is relevant for suspected bowel ischaemia needs further evaluation.

In spite of the diagnostic accuracy of the test no apparent improvement in survival was observed in the present study. Of the three patients who were in a condition to benefit from revascularization at the time when the D-dimer level was assessed, only one survived. The potential survival benefit needs to be investigated in a larger study. There was no death among the four patients with bowel ischaemia due to strangulation and the patient with an AAA. These five patients may have benefited from early laparotomy.

A negative test for D-dimer had both high sensitivity and high predictive value in the diagnosis of acute thromboembolic occlusion of the SMA. In this preliminary study only patients with clinical suspicion of a sudden mesenteric arterial occlusion were examined. Four patients with strangulation of the intestine due to adhesions also had a raised D-dimer level. Both the diagnosis of acute SMA occlusion and intestinal strangulation are urgent where the survival of the patient is at stake. The D-dimer test is now widely available in emergency wards and it could be a practical aid in decision making for urgent surgery. To further evaluate its specificity, and the predictive value of a positive test, a larger study is warranted.

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Original article

Value of intraoperative duplex imaging during supervised carotid endarterectomy

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Background: For overall benefit, carotid endarterectomy requires low perioperative morbidity and mortality rates. Carotid thrombosis is usually secondary to technical error, which may be related to the experience of the operator. In this retrospective study the clinical and technical outcome of carotid endarterectomies performed by one consultant and five trainees were compared.
Methods: Some 149 patients underwent carotid endarterectomy; 89 were operated on by the consultant and 60 by trainees. Intraoperative duplex imaging of the carotid repair was performed before wound closure, and re-exploration was carried out when there was a residual severe stenosis associated with an intimal flap.
Results: There was no significant difference in clinical outcome between operations done by consultant or trainees. There was a significant increase in the number of stenoses, kinks and flaps in carotid endarterectomies performed by trainees compared with those of the consultant both before ($\chi^2 = 12\cdot0$, 1 d.f., $P < 0\cdot001$) and after ($\chi^2 = 10\cdot1$, 1 d.f., $P < 0\cdot001$) correction.
Conclusion: Intraoperative duplex imaging may facilitate training by providing an objective assessment of the quality of the operation.

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Introduction

A low rate of perioperative complications is important if patients are to derive long-term benefit from carotid endarterectomy. Studies have reported 30-day stroke and death rates of 6·5 and 7 per cent for symptomatic patients and 2·3 per cent for asymptomatic patients undergoing carotid endarterectomy[1–3]. A more recent systematic review of carotid endarterectomy involving more than 50 hospitals reported a range of complication rates that were attributed to variations in study methodology[4,5]. The increasing number of carotid endarterectomies performed over the past decade has emphasized the importance of documenting and auditing the complication rates of surgical teams, and particularly the effect of allowing surgical trainees to perform the operation.

Most studies have shown that adequately supervised trainees performing carotid endarterectomy do not adversely influence outcome[6–10], although an earlier audit conducted by the Vascular Surgical Society reported that the seniority of the surgeon was an independent risk factor for stroke[11]. Perioperative stroke is related to operative technical error and could be avoided. Completion imaging using angioscopy demonstrated no significant difference in the incidence of retained thrombus or intimal flaps after carotid endarterectomy performed by either trainees or consultants[10]. Intraoperative duplex imaging has shown that residual stenoses and kinks at the distal endarterectomy site may influence clinical outcome and can lead to early restenosis[12].

In the present study, completion duplex imaging was used to audit the technical outcome of carotid endarterectomy in procedures performed by a consultant and five trainees.

Patients and methods

A consecutive series of 149 patients who underwent carotid endarterectomy between June 1995 and November 1997 were entered into this non-randomized study. Eighty-nine endarterectomies were performed by the consultant and the remaining 60 by the five trainees (median 13, range 6–20 operations). There was some selection of cases in terms of technical difficulty or clinically high-risk patients; these were performed by the consultant and included patients who presented with multiple transient ischaemic attacks (TIAs) (three), those where the distal end of the stenosis was not seen clearly on duplex imaging (two), those where the lesion was associated with free-floating thrombus (one) and those where the bifurcation was high (one). Patient demographics including age, sex and presenting symptoms are shown in *Table 1*, together with information on operative variables. There was no significant difference in the number of symptomatic and asymptomatic patients between the two groups ($\chi^2 = 0{\cdot}02$, 1 d.f., $P = 0{\cdot}88$).

Carotid endarterectomy was performed under general anaesthesia with selective intraluminal shunting based on perioperative transcranial Doppler monitoring. The consultant supervised and assisted in all operations done by trainees. Following conventional endarterectomy, the arteriotomy was closed primarily by continuous suture, with selective patching reserved for small-calibre arteries (less than 4 mm in external diameter). A similar number of patients in each group had a shunt ($\chi^2 = 1{\cdot}82$, 1 d.f., $P = 0{\cdot}18$) or a patch ($\chi^2 = 0{\cdot}09$, 1 d.f., $P = 0{\cdot}76$) (*Table 1*).

On restoration of blood flow, the endarterectomy site was examined with duplex ultrasonography. The intraoperative transducer (5–10 MHz; ATL, Letchworth, UK) was placed in a sterile plastic sleeve containing acoustic gel and was then coupled directly to the vessel with the aid of saline. B-mode images and blood velocity spectra along the length of the endarterectomy were recorded to hard disk for subsequent analysis. The completion study took less than 5 min to perform for each patient.

Table 1 Demographics and operative variables in 149 patients having carotid endarterectomy

	Consultant	Trainees
No. of patients	89	60
Mean(s.d.) age (years)	65(8)	64(9)
Sex ratio (M : F)	58 : 31	40 : 20
Presenting symptoms		
Stroke	19	18
TIA	30	12
Amaurosis fugax	23	15
Global ischaemia	3	5
Asymptomatic	14	10
Operative variables		
Shunting	36	31
Patching	9	7

TIA, transient ischaemic attack

Blood velocity data from the common and internal carotid arteries were used to grade the endarterectomized artery into four categories based on conventional duplex criteria[13]. The categories were: no significant disease (less than 50 per cent), moderate stenosis (50–69 per cent), severe stenosis (70–99 per cent) and occluded (100 per cent). B-mode images were analysed for detection of anatomical defects. This included identification of a residual intimal shelf which was defined as an intima–media thickness greater than 1·5 mm and an intimal flap that was a partially detached intima–media complex. A vessel kink was classified as a change in course of the vessel greater than 50°. Fronds were small, mobile, adherent threads seen on the endarterectomy surface.

Statistical analysis of the data was performed with the χ^2 test, with Yates' correction for analyses with small sample numbers.

Results

Clinical outcome

The rate of stroke and death was 3 per cent in both groups (three of 89, and two of 60), with an overall neurological event rate of 2 per cent (two of 89) for operations done by the consultant and 5 per cent (three of 60) for those performed by trainees. There was, however, no significant difference between the two groups in the stroke and death rate ($\chi^2 = 0{\cdot}20$, 1 d.f., $P = 0{\cdot}65$) or overall neurological event rate ($\chi^2 = 0{\cdot}0$, 1 d.f., $P = 1{\cdot}0$).

One patient suffered a stroke 48 h after operation by consultant and subsequently died, and there was one further death; both deaths were a result of myocardial infarction. Completion duplex and repeat postoperative duplex imaging demonstrated widely patent vessels in both patients. There was one postoperative TIA in the consultant operation group that occurred in a patient who had a primary patch. Postoperative duplex imaging showed a widely patent internal carotid artery with no significant abnormality at the endarterectomy site.

After operation by trainees, two patients suffered a stroke, one of whom also had a myocardial infarction and subsequently died. Duplex imaging demonstrated widely patent carotid arteries in both of these patients. There was a single TIA in the trainee operation group in a patient with recognized clamp damage on postoperative duplex imaging.

Intraoperative duplex imaging

Nine residual stenoses (10 per cent) were detected at the distal extent of the endarterectomy after consultant operation and 13 (22 per cent) in the trainee operation group; the increase was not statistically significant ($\chi^2 = 3{\cdot}8$, 1 d.f., $P = 0{\cdot}051$).

Reintervention based on duplex findings was performed when a severe stenosis (greater than 70 per cent) was associated with an intimal flap. In the consultant operation group five of the nine stenoses were severe; three of these were re-explored and corrected. In the trainee operation group, eight stenoses were severe and four were re-explored and corrected. After correction, the rate of residual stenosis was 7 per cent (six of 89) and 15 per cent (nine of 60) for consultant and trainees respectively. This difference was not statistically significant.

Nine intimal flaps and 13 kinks were detected at the distal extent of the endarterectomy in the operations done by trainees, giving a total anatomical abnormality rate of 37 per cent (22 of 60). This was significantly greater than the rate in operations performed by the consultant, which was 20 per cent (18 of 89; $\chi^2 = 4{\cdot}9$, 1 d.f., $P = 0{\cdot}03$).

Following correction of four abnormalities in the trainee operation group and three in the consultant operation group, the residual abnormality rate reduced to 30 and 17 per cent respectively. The final residual anatomical abnormality rate was not significantly different between the two groups ($\chi^2 = 3{\cdot}59$, 1 d.f., $P = 0{\cdot}06$).

The cumulative residual abnormality rate (stenosis, kink or flap – carotid arteries affected by one or more of these abnormalities) was 48 per cent (29 of 60) for the trainee operation group, which was significantly greater than the 21 per cent (19 of 89) for the consultant operation group ($\chi^2 = 12{\cdot}0$, 1 d.f., $P < 0{\cdot}001$). Following correction of four carotid arteries in the trainee operation group and three consultant operations, the number of vessels with a residual defect was still significantly higher for trainees ($\chi^2 = 10{\cdot}1$, 1 d.f., $P < 0{\cdot}001$).

Discussion

Several studies have shown that vascular trainees can perform carotid endarterectomy with acceptably low perioperative morbidity and mortality rates[6–10]. In studies where consultants' results were slightly worse than those of trainees, the case mix was skewed, with more technically challenging operations being done by consultants[9]. In the present series, difficult operations were also allocated to the consultant, but there was no significant difference in clinical outcome.

It is accepted that meticulous attention to detail, the use of completion imaging and correction of technical errors all contribute to low perioperative complication rates. It could therefore be expected from these findings that technical outcome would parallel clinical outcome. This was the case in a study using completion angioscopy that reported similar technical error rates in endarterectomies performed by trainees and consultants[10]. However, with intraoperative duplex imaging an increased incidence of technical problems was found in operations done by trainees. The difference in findings may be attributed to the completion technique. Angioscopy is performed before restoration of blood flow and, while having the advantage of identifying retained thrombus, it is unable to demonstrate clamp damage or intimal flaps, which may be visible only when blood flow is restored. Intraoperative duplex imaging is performed after restoration of blood flow and may therefore provide improved visualization of all technical abnormalities.

Completion duplex imaging has been useful not only for identifying residual defects that are haemodynamically significant, but also for modifying surgical technique. These modifications include resection of the proximal end of the plaque to ensure that there is no residual flap and modification of proximal and distal clamping to reduce the incidence of clamp damage. Similarly, the immediate feedback provided by completion duplex imaging enables trainees to appreciate modifications in operative technique and improvement in technical outcome.

Operative morbidity and mortality rates decrease with both cumulative experience and the number of carotid endarterectomies performed each year[14]. In the present study there was no significant difference in clinical outcome between consultant and trainees but there was an increased incidence of technical problems for the trainees. Intraoperative duplex imaging may facilitate training by providing an objective assessment of the quality of the endarterectomy.

Acknowledgements

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Original article

Nitric oxide prevents intestinal mitochondrial dysfunction induced by surgical stress

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Background: The intestine is highly susceptible to free radical-induced damage and earlier work has shown that surgical stress induces generation of oxygen free radicals in enterocytes, resulting in intestinal damage along with changes in mitochondrial structure and function. Nitric oxide is an important mediator of gastrointestinal function and this study looked at the effect of nitric oxide on surgical stress-induced intestinal mitochondrial alterations.

Methods: Controls and rats pretreated with the nitric oxide donor L-arginine were subjected to surgical stress by opening the abdominal wall and handling the intestine. Enterocytes were isolated, mitochondria prepared and the protection offered by L-arginine against damage due to surgical stress was determined. Protection to structural as well as functional aspects of mitochondria was examined.

Results: Mild handling of the intestine affected the enterocyte mitochondrial structure as assessed by lipid composition and electron microscopy. Mitochondria were also functionally impaired with altered calcium flux and decreased respiratory control ratio. Pretreatment with the nitric oxide synthase substrate L-arginine prevented these damaging effects of surgical stress. Protection with arginine was abolished by the nitric oxide synthase inhibitor N^G-nitro-L-arginine methyl ester, indicating the role of nitric oxide.

Conclusion: Surgical stress in the small intestine can affect enterocyte mitochondrial structure and function. These damaging effects can be prevented by nitric oxide, an important modulator of cellular function.

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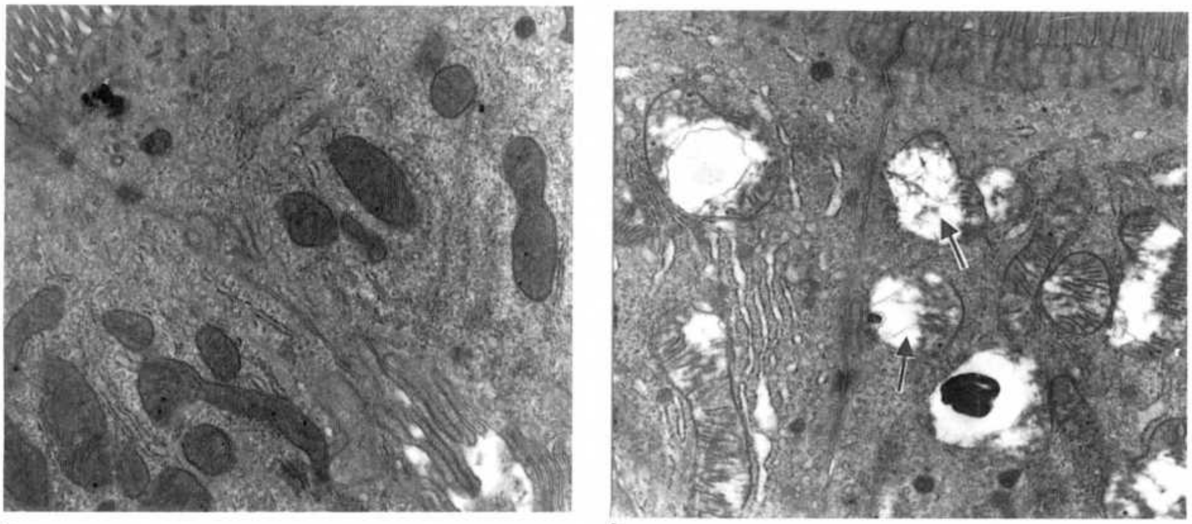
Introduction

The gut is now recognized to play an important role in postoperative complications such as the systemic inflammatory response syndrome and multiple organ failure[1]. Loss of gut barrier function and increased gut permeability have been reported after surgery[2], and previous work has shown that surgical stress can induce widening of intercellular spaces, increased intestinal permeability and oxidative stress in enterocytes[2]. Free radicals have been implicated in surgical stress-induced damage[3,4] and mitochondria are an important source, as well as a target, of free radicals at the cellular level. Mitochondrial dysfunction may thus play a role in the initiation of cellular damage.

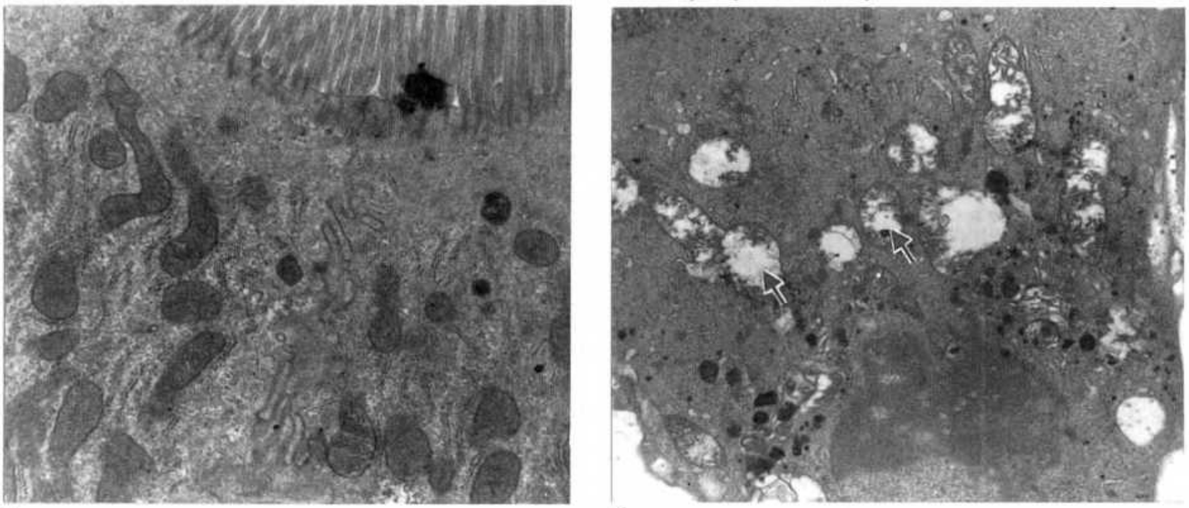
Nitric oxide is a biological molecule whose functions have come under intense investigation. Nitric oxide is an important mediator of mucosal defence, influencing certain factors such as mucus secretion, mucosal blood flow and ulcer repair[5]. L-Arginine administration reverses the deleterious effect of nitric oxide synthase inhibition on mucosal barrier function[6] and earlier work has shown that oxidative stress-induced damage to the intestine following ischaemia–reperfusion injury can be ameliorated by the luminal presence of nitric oxide donor[7]. The primary objective of this study was to examine the effect of L-arginine pretreatment on surgical stress-induced alterations to intestinal mitochondrial structure and function.

Materials and methods

Adenosine 5′-triphosphate (ATP), adenosine diphosphate, bovine serum albumin, dichlorophenol indophenol, ethyleneglycol-bis(β-aminoethyl ether)-N,N,N′,N′-tetra-acetic acid (EGTA), iodonitrotetrazolium violet, nicotinamide adenine dinucleotide (NAD), its reduced form (NADH), nicotinamide adenine dinucleotide phosphate (NADP), N^G-nitro-L-arginine methyl ester (L-NAME) and succinic acid were obtained from Sigma Chemical (St



a Control **b** Surgically stressed only



c L-arginine pretreated **d** L-arginine plus L-NAME pretreated

Fig. 1 Electron micrographs of small intestinal mitochondria from controls and rats subjected to surgical stress in the presence and absence of L-arginine and N^G-nitro-L-arginine methyl ester (L-NAME). The control has normal mitochondria (**a**). Surgically stressed cells have swollen mitochondria (arrows) with loss of cristae, dilated rough endoplasmic reticulum and myelin figures (**b**). L-Arginine pretreatment before surgical stress results in unaltered mitochondria with well preserved cristae (**c**), while surgically stressed epithelial cells pretreated with L-arginine and L-NAME have dilated mitochondria (arrows), intracytoplasmic vacuoles and lipid droplets (**d**). (Original magnification × 15 000)

Louis, Missouri, USA). All other chemicals used were of analytical grade.

Animals

Adult Wistar rats of both sexes (200–250 g), exposed to a daily 12-h light–dark cycle and fed water and rat chow *ad libitum*, were used. The rats were divided randomly into four groups ($n = 6$): control (arginine pretreatment without surgery), surgical stress (intestinal handling), arginine (surgical stress after arginine pretreatment) and L-NAME (surgical stress after arginine pretreatment in presence of L-NAME). This study was approved by the Animal Experimentation Ethics Committee of the institution.

Pretreatment

Rats were given an intraperitoneal injection of 0·3 g per kg body-weight of L-arginine[8,9] 30 min before surgery. L-NAME 0·1 mg/ml was administered for 9 days in drinking

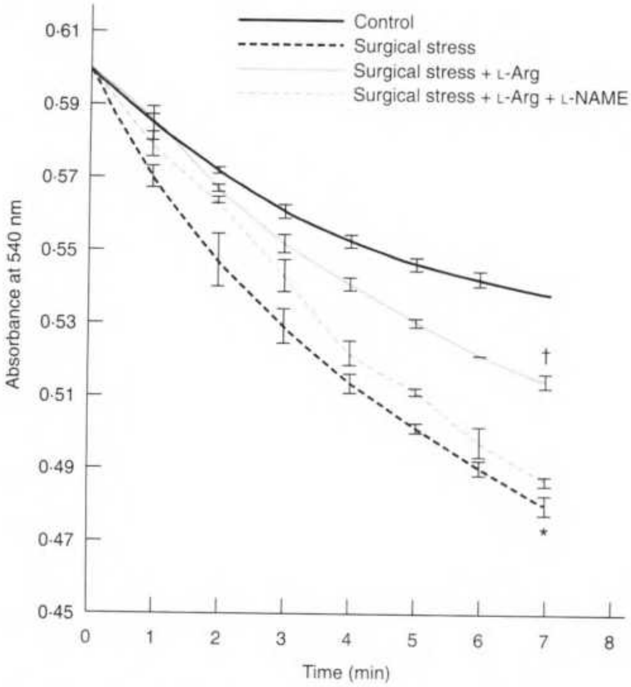
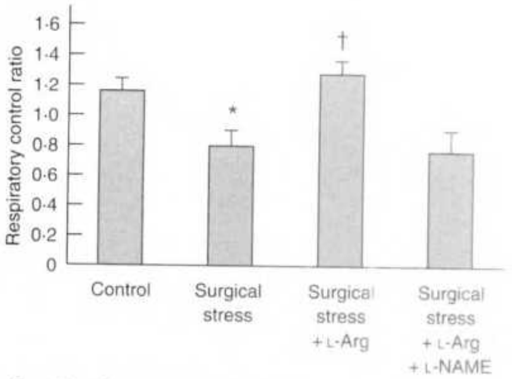
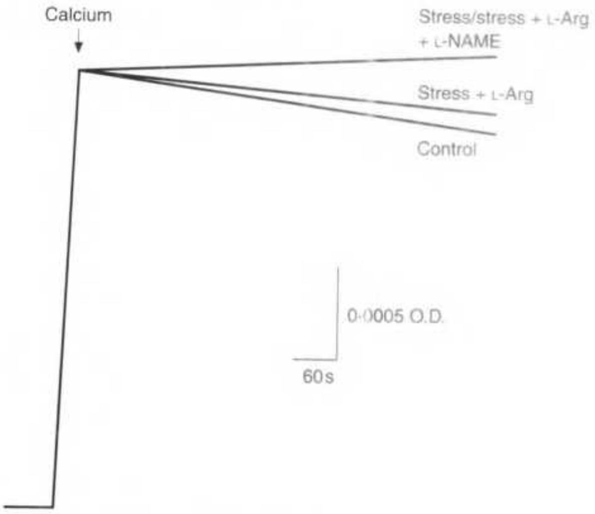


Fig. 2 Mitochondrial swelling in enterocytes isolated from controls and rats that were surgically stressed with or without pretreatment with L-arginine or L-arginine + N^G-nitro-L-arginine methyl ester (L-NAME). Values are mean(s.d.). $*P < 0.01$ *versus* control, $\dagger P < 0.01$ *versus* surgical stress (Student's *t* test)



a Respiratory control ratios



b Calcium flux in mitochondria

Fig. 3 **a** Mean(s.d.) respiratory control ratio of enterocyte mitochondria isolated from controls and rats that were surgically stressed with or without pretreatment with L-arginine or L-arginine + N^G-nitro-L-arginine methyl ester (L-NAME). $*P < 0.01$ *versus* control, $\dagger P < 0.01$ *versus* surgical stress (Student's *t* test). **b** Representative traces of the calcium flux in enterocyte mitochondria. Calcium 10 µmol/l was added as indicated. O.D., optical density

water. Control animals were injected with the same dose of L-arginine 90 min before killing the animal without surgery.

Induction of surgical stress

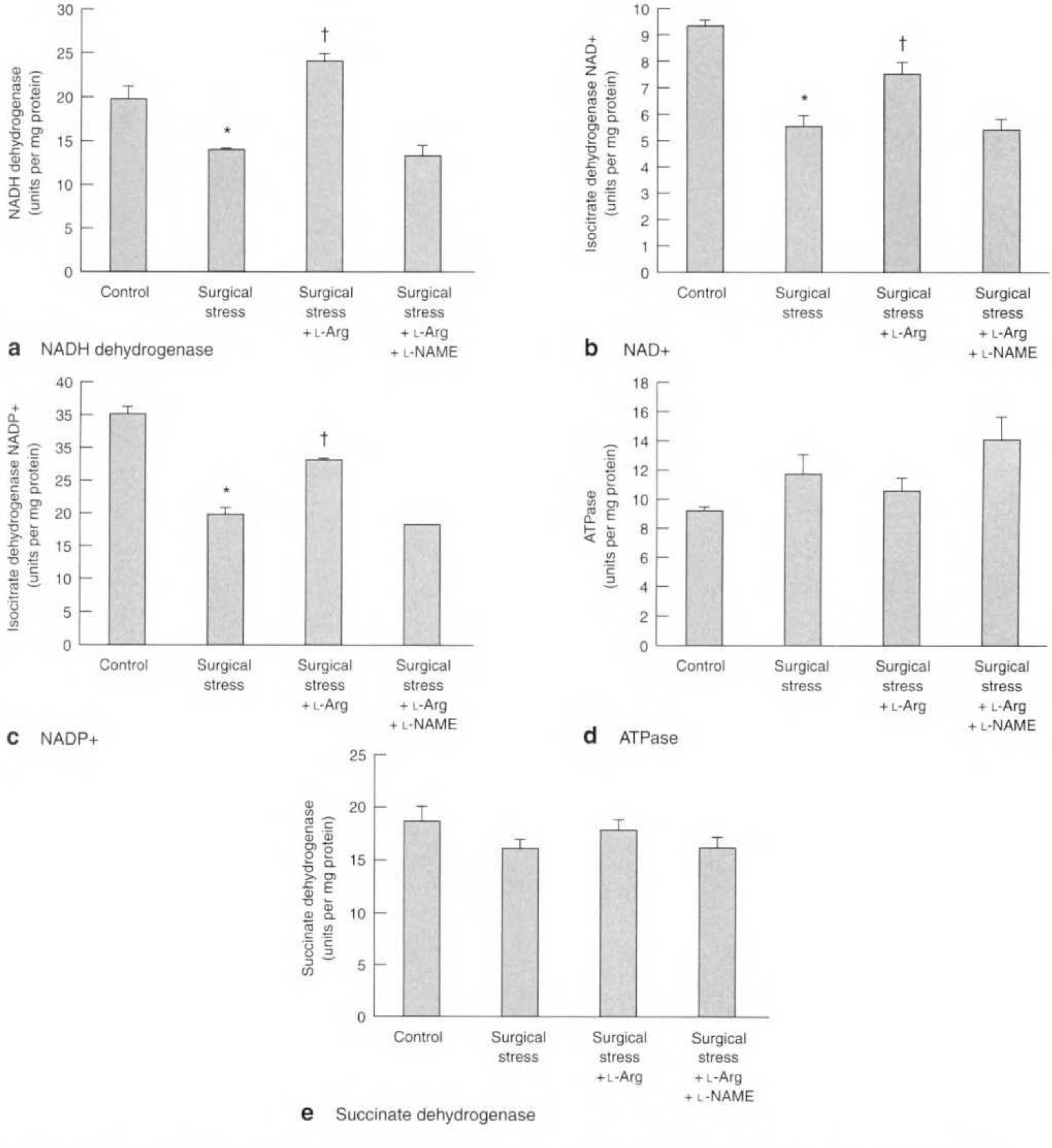
Surgical stress was induced as described[2]. Mitochondria were prepared from isolated enterocytes[10] from control and surgically stressed rats, 60 min after surgical stress, in the presence and absence of L-arginine and L-NAME. It has been shown that the damage is maximal by 60 min after stress in this model[2]. For histological studies, animals were killed, the intestine was removed and intestinal tissue samples were processed for ultrastuctural studies using a Philips EM201C electron microscope (Philips, Eindhoven, Netherlands).

Assessment of mitochondrial function

Mitochondrial oxygen uptake was determined using a Clark type electrode as described previously[11] and calcium flux was quantified by measuring changes in the absorption spectrum of Arsenazo III at 675–685 nm[12]. Mitochondrial enzymes were measured spectrophotometrically as described before[13–17] and swelling determined by the decrease in absorbance at 540 nm up to 10 min[18].

Lipid analysis

Mitochondrial lipids were extracted by the method of Bligh and Dyer[19]. Lipids were separated by thin layer chromatography. Cholesterol[20], diacylglycerol and triacylglycerol[21] were quantified as described elsewhere. Free fatty acids were methylated and quantified by gas chromatography.



a NADH dehydrogenase

b NAD+

c NADP+

d ATPase

e Succinate dehydrogenase

Fig. 4 Mean(s.d.) activity of the mitochondrial enzymes reduced nicotinamide adenine dinucleotide (NADH) dehydrogenase (**a**), isocitrate dehydrogenase nicotinamide adenine dinucleotide (NAD)+ (**b**), isocitrate dehydrogenase nicotinamide adenine dinucleotide phosphate (NADP)+ (**c**), adenosine 5′-triphosphatase (ATPase) (**d**) and succinate dehydrogenase (**e**) in enterocytes isolated from controls and rats that underwent surgical stress with or without pretreatment with L-arginine or L-arginine + N^G-nitro-L-arginine methyl ester (L-NAME). $^*P < 0{\cdot}01$ *versus* control, $^\dagger P < 0{\cdot}01$ *versus* surgical stress (Student's *t* test)

Phospholipids were separated and then quantified by phosphate estimation after acid hydrolysis[22–24].

Statistical analysis

Values are mean(s.d). Statistical analysis was by Student's *t* test.

Results

On ultrastructural examination, control small intestinal mitochondria appeared normal (*Fig. 1a*). Surgically stressed epithelial cells (*Fig. 1b*) showed marked swelling of mitochondria with distortion of cristae in areas with or without paracellular widening. Myelin figures were seen in the intercellular spaces, within the mitochondria and also in the subepithelial connective tissue. Pretreatment with the nitric oxide donor L-arginine (*Fig. 1c*) protected against mitochondrial damage and paracellular widening. Inclusion of the nitric oxide synthase inhibitor L-NAME along with arginine inhibited this protective effect of L-arginine (*Fig. 1d*). These histological findings were confirmed by spectrophotometric measurement of mitochondrial swelling, which showed an increase in mitochondrial swelling 60 min after surgical stress. This was prevented by L-arginine pretreatment. Inclusion of L-NAME reversed the protective effect of arginine pretreatment (*Fig. 2*).

Studies of mitochondrial respiration showed that L-arginine pretreatment protected against the decrease in mitochondrial respiratory control ratio seen after surgical stress (*Fig. 3a*). This protection was also prevented by L-NAME pretreatment.

Mitochondrial calcium flux studies showed an uptake of calcium in control mitochondria, which was abolished in mitochondria prepared from intestine 60 min after surgical stress. Pretreatment with L-arginine restored calcium influx, but not when L-NAME was also given (*Fig. 3b*).

Surgical stress induced a decrease in the activity of NADH dehydrogenase and isocitrate dehydrogenase (both NAD and NADP specific). This decrease was prevented by L-arginine pretreatment, although inclusion of L-NAME reversed the protection (*Fig. 4*). There was no change in the activity of succinate dehydrogenase or ATPase following surgical stress.

Table 1 Enterocyte mitochondrial phospholipid composition in controls and after surgical stress in the presence and absence of L-arginine and N^G-nitro-L-arginine methyl ester

Phospholipid	Control	Surgical stress	Surgical stress + L-Arg	Surgical stress + L-Arg + L-NAME
Phosphatidyl choline (nmol per mg protein)	41·48(3·14)	18·78(0·93)*	40·28(3·16)†	23·95(2·30)
Phosphatidyl ethanolamine (nmol per mg protein)	21·32(1·75)	10·31(0·90)*	23·20(2·14)†	12·17(1·35)
Sphingomyelin (nmol per mg protein)	11·00(0·91)	10·66(0·27)	11·87(0·68)	12·52(1·18)
Phosphatidyl serine/phosphatidyl inositol (nmol per mg protein)	11·08(1·34)	10·19(1·14)	11·29(0·76)	12·56(1·03)
Lysophosphatidyl choline (nmol per mg protein)	3·58(0·21)	8·01(0·30)*	4·00(0·50)†	9·75(1·40)
Lysophosphatidyl ethanolamine (nmol per mg protein)	3·76(0·41)	10·16(1·35)*	4·03(0·43)†	11·80(0·81)
Phosphatidic acid (nmol per mg protein)	15·50(1·34)	21·64(1·28)*	13·96(1·40)†	27·57(2·22)

Values are mean(s.d.). L-NAME, N^G-nitro-L-arginine methyl ester. *$P < 0·01$ *versus* control, †$P < 0·01$ *versus* surgical stress (Student's *t* test)

Table 2 Individual free fatty acids of enterocyte mitochondria from controls and after surgical stress in the presence and absence of L-arginine and N^G-nitro-L-arginine methyl ester

Free fatty acid	Control	Surgical stress	Surgical stress + L-Arg	Surgical stress + L-Arg + L-NAME
Palmitic acid (nmol per mg protein)	10·50(0·52)	29·32(3·32)*	12·54(1·09)†	33·00(3·11)
Stearic + oleic acid (nmol per mg protein)	6·50(0·64)	25·00(2·56)*	10·96(0·69)†	26·00(2·55)
Lauric acid (nmol per mg protein)	1·12(0·14)	5·87(0·93)*	1·13(0·15)†	2·40(0·28)
Linoleic acid (nmol per mg protein)	3·79(0·56)	7·10(0·86)*	4·20(0·55)†	6·44(0·77)
Arachidonic acid (nmol per mg protein)	2·13(0·88)	4·63(0·85)*	1·22(0·05)†	5·56(0·55)
Total free fatty acids (nmol per mg protein)	24·04(0·54)	71·92(1·72)	30·05(0·50)	73·40(1·45)

Values are mean(s.d.). L-NAME, N^G-nitro-L-arginine methyl ester. *$P < 0·01$ *versus* control, †$P < 0·01$ *versus* surgical stress (Student's *t* test)

Lipid analysis of the mitochondria showed alteration in the phospholipid composition, especially a decrease in phosphatidyl choline and phosphatidyl ethanolamine accompanied by an increase in lysophosphatidyl choline and lysophosphatidyl ethanolamine (*Table 1*). An increase in free fatty acids was also seen in surgically stressed mitochondria (*Table 2*). There was also an increase in the level of phosphatidic acid in surgically stressed mitochondria (*Table 1*). These phospholipid alterations and increase in free fatty acids were prevented by L-arginine pretreatment, an effect that was abolished by L-NAME administration. There was no change in the neutral lipid composition in the stressed mitochondria (data not shown).

Discussion

In recent years, the gastrointestinal tract has assumed importance in the development of sepsis, systemic immune response syndrome and multiple organ failure[1]. It was shown previously that surgical stress leads to activation of the superoxide generating enzyme xanthine oxidase in the small intestine and that this causes considerable damage to intestinal structure and function[2,11]. Mitochondria are important subcellular organelles involved in energy production that are susceptible to oxidative stress and have been shown to be damaged in sepsis[25].

Nitric oxide, produced by the enzyme nitric oxide synthase[26], ameliorates vasoconstriction in the small intestine during bacteraemia via an L-arginine pathway[27]. Nitric oxide prevents the intestinal mitochondrial damage that occurs during ischaemia–reperfusion injury to the intestine[7] and, in the present study, the effect of L-arginine on surgical stress-induced alterations to the intestinal mitochondria was examined. Arginine pretreatment protected against mitochondrial swelling and these animals had morphologically normal mitochondria. To confirm that protection was due to the synthesis of nitric oxide, experiments were also done in rats given the nitric oxide synthase inhibitor L-NAME together with arginine. This prevented the protection, proving the role of nitric oxide in this process. The histological findings were confirmed by spectrophotometric measurements of mitochondrial swelling.

Mitochondrial function has been shown to be affected after sepsis[28,29] and it has been observed that surgical stress also affects intestinal mitochondrial function by decreasing the respiratory control ratio and by altering mitochondrial enzyme activities, such as NADH dehydrogenase, that form part of the electron transport chain. Nitric oxide prevents the decrease in respiratory control ratio during apoptosis of HT-29 cells[30] and ischaemia–reperfusion injury in the intestine[7]. Mito-chondria play an important role in maintaining cellular calcium[31] and mitochondrial calcium uptake affected by surgical stress was restored with arginine pretreatment.

Lipids play a crucial role in mitochondrial function[32]. Surgical stress resulted in mitochondrial phospholipid degradation, especially phosphatidyl ethanolamine and phosphatidyl choline, and an increase in phosphatidic acid, suggesting activation of phospholipase D. Earlier work has shown the presence of phospholipase D in intestinal mitochondria, which can be activated by oxygen free radicals[33]. The lipid profile also suggested activation of phospholipase A_2, the cytosolic form that can be activated by alterations in cytosolic calcium[34]. Since mitochondrial calcium uptake is affected, cytosolic calcium levels are probably altered in the enterocytes during surgical stress, which results in phopholipase A_2 activation, thereby generating lysophospholipids and free fatty acids. These lipid alterations are prevented by L-arginine pretreatment. Nitric oxide can act as an antioxidant in membranes[35] and prevent cellular damage due to hydrogen peroxide[36]. Nitric oxide also reversibly inhibits xanthine oxidase, a major source of free radicals[37].

In conclusion, this study has shown structural and functional alterations to enterocyte mitochondria during surgical stress which can be prevented by prior administration of the nitric oxide donor, L-arginine. The exact mechanism by which nitric oxide offers protection under such conditions may involve multiple pathways and is under investigation.

Acknowledgements

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Original article

Outcome and late functional results after anastomotic leakage following mesorectal excision for rectal cancer

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Background: Few studies have evaluated the long-term functional outcome after anastomotic leakage in the treatment of rectal cancer.

Methods: Between 1993 and 1998, 147 patients were admitted with resectable rectal carcinoma, and 92 underwent low anterior resection (LAR). Seventeen patients (18 per cent) developed clinical anastomotic leakage. The functional outcome of 11 of 12 patients, in whom the stoma was subsequently closed and bowel continuity was restored without stricture, was compared with that of 11 matched patients who had undergone LAR without leakage. Anorectal manovolumetry and symptom scoring on visual analogue scales were done 12–48 months after stoma closure.

Results: Nine patients made an uneventful recovery after the initial treatment of anastomotic leakage. Eight developed serious septic complications, four of whom had a pelvic abscess, but there was no death. Five patients had chronic complications that precluded closure of the stoma. Patients who had experienced leakage showed reduced neorectal capacity (120 *versus* 180 ml; $P = 0.04$), more evacuation problems ($P = 0.02$), and a trend towards more faecal urgency ($P = 0.09$) and incontinence ($P = 0.06$) than control patients.

Conclusion: Stoma closure was not possible in five of 17 patients who had experienced anastomotic leakage. Patients who had the stoma closed had impaired long-term anorectal function compared with control patients without leakage.

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Introduction

Anastomotic leakage is the most significant early complication after rectal resection with a stapled anastomosis. Since the introduction of mesorectal excision as the standard treatment of rectal cancer in this hospital in 1993, a greater proportion of patients has had sphincter-saving operations than before and more patients have a low anastomosis. As a consequence the frequency of anastomotic leakage has increased. Leakage may cause serious early morbidity, and also death, and may affect the long-term outcome and functional result. Several aspects of anastomotic leakage have been studied extensively[1–15], but few series have considered late functional outcome[16]. Most studies have reported only whether or not closure of the stoma was possible[2,5,6,9,12].

The aim of this study was to review the profile of early postoperative complications after the introduction of mesorectal excision in this department and to evaluate the long-term functional results in patients with anastomotic leakage.

Patients and methods

During a 5-year period (1993–1998), 199 patients were admitted with rectal cancer at or below 15 cm from the anal verge; low anterior resection (LAR) was performed in 92 patients (*Table 1*). The standard operative technique included total mesorectal excision in patients with tumours in the lower and mid rectum, and partial mesorectal excision with transection of the mesorectum at least 5 cm distal to the tumour in patients with cancer of the upper rectum. In the majority of patients the ascending branch of the left colic artery was preserved and the sigmoid colon was used for the anastomosis, which was constructed by a double-stapling technique. A defunctioning stoma was constructed as preferred by the surgeon, in most cases because of a low

Table 1 Operations performed in 199 patients admitted with rectal cancer

	No. of patients
Low anterior resection	92
Abdominoperineal resection	44
Hartmann procedure	8
Local resection	3
Total no. of rectal resections	147

anastomosis. Details of the primary operation performed are given in *Table 2*.

All relevant data from the operation, postoperative course and follow-up examinations at 3 months and every 6 months thereafter were recorded prospectively. Follow-up included endoscopy in all cases and rectal radiography if leakage was suspected.

In 17 patients (18 per cent) who developed clinical signs of anastomotic leakage, the diagnosis was confirmed by radiography ($n = 14$), late endoscopy ($n = 1$), the occurrence of rectovaginal fistula ($n = 1$) or emergency laparotomy ($n = 1$). There were nine women and eight men in this group with a median age of 70 (range 45–80) years. The anastomoses were located 3–10 (median 5) cm above the anal verge. Histopathological examination showed tumour stage pT_4 in one, pT_3 in ten and pT_2 or pT_1 in six patients; four patients had lymph node metastasis (Dukes' C).

For evaluation of long-term results, 11 of 12 patients treated successfully for leakage, in whom bowel continuity was restored and there was no evidence of marked stricture formation, were compared with 11 patients who had undergone LAR without any sign of anastomotic leakage. The two groups were closely matched (*Table 3*).

Anal manometry was performed 12–48 months after closure of the stoma, using a stationary pull-through technique with the patient in the left lateral position. A water-perfused 4-mm catheter with four side holes was withdrawn in increments of 0·5 cm through the anal canal, and the side hole pressures were recorded on a PC Polygraph HR®, version 5.06 C2, series P 1775, with software Polygram for DOS (Medtronic Functional Diagnostics, Skovlunde, Denmark). The median value of maximum resting pressures recorded in each channel during pull-through was calculated. The procedure was then repeated and the maximum pressure increase during squeeze effort was recorded at each station. The median value of these pressures was calculated and defined as the maximum squeeze pressure.

Rectal volumetry was performed after manometry. A Zinetics® natural rubber latex anorectal balloon (Zinetics Medical, Salt Lake City, Utah, USA) was placed in the distal rectum and inflated with air in 10-ml increments. The volumes for initial sensation, urge to defaecate and the maximum tolerable volume were recorded.

Table 2 Operative details in 92 patients who underwent low anterior resection

	Total	Leakage
Low anterior resection	92	17 (18)
Defunctioning stoma	29	5 (17)
No defunctioning stoma	63	12 (19)
Total mesorectal excision	47	13 (28)
Partial mesorectal excision	45	4 (9)
End-to-end anastomosis	78	14 (18)
Side-to-end without reservoir	11	2 (18)
Colonic pouch	3	1 (33)

Values in parentheses are percentages

Table 3 Comparison of patients who developed anastomotic leakage and control patients

	Leakage ($n = 11$)	Controls ($n = 11$)
Sex ratio (M : F)	4 : 7	7 : 4
Age (years)*	60 (45–77)	65 (44–73)
Tumour height (cm)*	9 (5–15)	11 (6–15)
Anastomosis level (cm)*	5 (3–9)	6 (3–9)
Total mesorectal excision	7	6
Partial mesorectal excision	4	5
End-to-end anastomosis	8	7
Side-to-end without reservoir	2	3
Colonic pouch	1	1
Time from operation or stoma closure (months)*	24 (12–60)	12 (12–24)

*Values are median (range)

The patients graded their symptoms and defaecatory function (urgency of defaecation, pain during defaecation, continence, ability to expel stool and feeling of complete evacuation) on 100-mm long visual analogue scales, in which a value of 100 represented normal function (absence of symptom). The questionnaires were completed together with the physician to avoid misunderstandings.

For evaluation of the results, patients with and without leakage were compared using the Mann–Whitney *U* test for statistical analysis. Two-sided $P < 0.05$ was accepted as significant. Data were collected and stored in a database (Microsoft Access® version 2.0; Microsoft Corporation, Redmond, Washington, USA) and all statistical analyses were performed with the Statistical Package for Social Sciences version 8.0 (SPSS, Chicago, Illinois, USA).

The study was performed according to the Helsinki declaration and approved by the regional ethics research committee. Informed consent was given by the patients.

Table 4 Anal and neorectal manovolumetry results in patients with and without anastomotic leakage after low anterior resection

	Leakage (*n*=11)	Controls (*n*=11)	*P**
Resting pressure (mmHg)	52 (43–82)	59 (30–90)	0·75
Squeeze pressure (mmHg)	105 (65–150)	115 (30–190)	0·81
Initial sensation (ml)	25 (20–50)	44 (10–100)	0·13
Urge to defaecate (ml)	70 (40–110)	100 (50–280)	0·07
Maximum tolerable volume (ml)	120 (70–230)	180 (80–280)	0·04
Bowel movements (per day)	2·5 (0·5–4)	2·5 (0·5–3)	0·33

Values are median (range). *Mann–Whitney *U* test

Table 5 Anal and neorectal function in patients with and without anastomotic leakage after low anterior resection

	Leakage (*n*=11)	Controls (*n*=11)	*P**
Ability to defer defaecation	50 (5–100)	86 (10–100)	0·09
Ability to expel stool	73 (14–97)	82 (7–100)	0·40
Feeling of complete evacuation	30 (5–75)	66 (4–100)	0·02
Painful defaecation	95 (73–100)	94 (5–100)	0·18
Incontinence	80 (45–100)	93 (50–100)	0·06

Values are median (range) symptom score on visual analogue scale. *Mann–Whitney *U* test

Results

Fourteen patients (15 per cent) had clinical signs of leakage during the hospital stay, and the diagnosis was established 2–12 (median 7) days after operation. Two patients developed severe peritonitis (day 2 and 6) whereas nine patients had only vague abdominal symptoms. Vaginal discharge was the only symptom in one and rectal bleeding the only symptom in two patients. Three patients (3 per cent) had no clinical signs of leakage during the hospital stay. Symptoms of rectovaginal fistula in one and anal discharge in the other two occurred 3–4 weeks after operation and the diagnosis was then confirmed.

Eleven patients were reoperated. Laparotomy with drainage and construction of a diverting stoma was performed in eight, and a Hartmann procedure in three patients. Five patients who had a diverting stoma at the primary operation were not reoperated, nor was one patient without a diverting stoma who had minor symptoms.

After the initial treatment of anastomotic leakage, eight patients developed serious septic complications and four required a second reoperation owing to the development of a pelvic abscess, but there was no death. Nine patients made an uneventful recovery. The hospital stay ranged from 19 to 104 (median 39) days.

After discharge from hospital, five patients had chronic anal discharge due to an infected perirectal cavity, two patients after a Hartmann procedure and three patients after reoperation with drainage. One patient also had local tumour recurrence and stricture. The discharge finally subsided in one patient, whereas it persisted and precluded closure of the diverting stoma in four patients. In addition, one patient with an uneventful recovery after a Hartmann procedure had a permanent stoma. Thus, five patients required a permanent stoma because of the anastomotic leakage. Three patients had a persisting perirectal cavity without discharge and two had a moderate anastomotic stricture necessitating dilatation; the stoma was closed in these patients.

Stoma closure was possible in 11 patients and was performed 3–22 (median 8) months after the primary operation. These patients and one patient observed without a diverting stoma were candidates for evaluation of late anorectal function. Eleven patients were examined; one elderly patient was excluded. The results of anorectal manovolumetry are presented in *Table 4*. Anal sphincter pressures in patients who had experienced leakage were no different from those in controls. However, these patients had a significantly lower maximum tolerable neorectal volume than controls. Patients who had anastomotic leakage experienced greater problems with complete evacuation, and a trend towards more urgency and faecal incontinence than controls (*Table 5*).

Four of five patients who had a defunctioning stoma at the primary operation had obvious signs of leakage during the hospital stay, whereas one patient had later onset of symptoms. All of these patients were observed initially without reoperation. Two developed serious septic complications, one of whom was reoperated 6 weeks after operation with conversion to a Hartmann procedure. Stoma closure was possible in only two patients in this group, whose late functional results were no better than those in patients without a primary defunctioning stoma.

Discussion

The most important findings in the present study were that one-third of the patients with anastomotic leakage required a permanent stoma owing to long-term complications that precluded closure, and that the functional results in patients who had a defunctioning stoma closed were inferior to those experienced by patients without anastomotic leakage.

Several studies have shown that closure of the stoma is not possible in 20–63 per cent of patients who experience anastomotic leakage following anterior resection[2,5,6,9,12,16]. A literature search has revealed only one previous study that evaluated long-term functional results in patients with anastomotic leakage and subsequent closure of the stoma. Hallböök and Sjödahl[16] found a reduction in neorectal reservoir function in patients with previous leakage compared with that in controls without leakage, reflected in impaired anorectal function. Frequent bowel movements, urgency of defaecation and faecal incontinence were more pronounced in patients who had experienced an anastomotic leak. The present study confirms that neorectal capacity is reduced after leakage, most probably owing to failure of healing by first intention with granulation tissue formation and consequent fibrosis[17]. None of the patients who had a stoma closed had a permanent stricture, but scarring and fibrosis probably resulted in a non-compliant neorectum with reduced capacity and motility. In this series neorectal capacity was measured using a rubber balloon. This type of balloon may expand in a longitudinal sausage-like fashion in a stiff fibrotic distal bowel and possibly give a falsely high volume. The true neorectal capacity might have been lower than was measured. Anal sphincter function was preserved, in keeping with observations by Hallböök and Sjödahl[16].

The main functional problem, reported by a majority of the patients, was inability to evacuate the bowel completely. The patients also experienced some degree of faecal urgency. Incontinence was not a major problem, but a few patients reported occasional incontinence to gas and loose stool. A significant difference in symptom scores was found only for evacuation problems; however, the number of patients was small.

Long-term results were evaluated at least 12 months after stoma closure in all patients. Functional results usually improve substantially during the first year after stoma closure and might improve even after the first year. The observation time was longer in the leakage group than the control group (*Table 3*) and, if the results improved after 12 months, the differences between the two groups might have been larger than those found.

Contrary to what is generally thought, a defunctioning stoma at the primary operation did not reduce the complications of anastomotic leakage. In this small series, the frequency of septic complications was the same, and the late functional results were no better than those in patients without a protective stoma. The initial consequences of leakage in defunctioned patients are usually less since such patients rarely need immediate reoperation, but larger studies are needed to investigate whether they really have a better long-term result than patients who are not defunctioned primarily.

The question arises of whether patients who suffer anastomotic leakage would do better with a permanent stoma. In the authors' experience, despite impaired anorectal function after leakage, most patients nevertheless would prefer intact anal function rather than a stoma. However, before closing a well functioning stoma, it seems logical to perform a physiological examination. If anal sphincter pressures or neorectal capacity are markedly reduced, impaired anorectal function would be expected and possibly such information should be given consideration. However, there are no studies that provide evidence of the predictive value of physiological tests before stoma closure.

In conclusion, anastomotic leakage is a serious complication that results in a permanent stoma in a proportion of patients. When healing of the anastomosis and restoration of bowel continuity is achieved, late anorectal function is impaired compared with that in patients without anastomotic leakage. More and larger studies are needed to evaluate long-term results in subgroups of patients with leakage.

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Original article

Scintigraphic assessment of colonic transit in women with slow-transit constipation arising *de novo* and following pelvic surgery or childbirth

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Background: Colonic transit has not been compared between patients with slow-transit constipation (STC) arising *de novo* (idiopathic) and those whose symptoms followed pelvic surgery or childbirth (acquired).
Methods: In 48 women, with either idiopathic ($n = 36$) or acquired ($n = 12$) STC, ^{111}In-radiolabelled diethylene-triamine penta-acetic acid colonic scintigraphy was performed to determine patterns of delay (generalized or left sided), the 'severity' of transit disturbance between subgroups, and the association with age or duration of symptoms. Results were compared with those in healthy women. Patterns of colonic transit disturbance were assessed using previously defined criteria. In those with a generalized delay, variables reflecting the overall rate of isotope progression throughout the colon were calculated: gradient of geometric centre of isotope progression and estimated evacuation time of the isotope.
Results: The pattern of transit delay was similar between the subgroups, but the 'severity' of the transit abnormality was significantly worse in those with chronic idiopathic symptoms. In the chronic idiopathic STC subgroup only, there was a significant correlation between both age and duration of symptoms and severity of transit disturbance.
Conclusion: This study demonstrates that differences in colonic transit exist between subgroups of patients with STC. These might be explained by differences in duration of symptoms or differences in aetiology.

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Introduction

Assessment of colonic transit in patients with intractable constipation[1–4] is essential to direct management strategy[5]. In patients with delayed transit (slow-transit constipation; STC), radionuclide studies have shown that the delay may be generalized throughout the large bowel[2,4] or localized to the left colon[2–4]. It is also clearly recognized that, in patients with the same pattern of transit disturbance, the progression of the radionuclide marker is slower in some than others, i.e. the transit disturbance is more 'severe'[2–4,6,7].

STC almost exclusively affects young females, and is usually referred to as being 'chronic' and 'idiopathic' in origin[8–10]. However, a second major subgroup of patients have symptoms subsequent to childbirth[11] or pelvic surgery (usually hysterectomy)[9,10,12]. While such apparent causality may be spurious, in that patients tend to anchor their decline to a particular event, this association is widely accepted[9–12]. Regardless of mode of onset, little attention has been paid to the natural history of this disorder, yet many patients complain of a progressive nature to their condition[8,10].

The aetiology of STC is probably heterogeneous, and clinical subgroups may represent different disease processes which variably affect the colon and other gastrointestinal and non-gastrointestinal organs. Contrary findings have been reported between patient subgroups with regard to the function of the upper gastrointestinal tract. A proportion of patients with chronic idiopathic STC are known to have a generalized intestinal, or panenteric, disorder[13,14], unlike patients whose symptoms follow childbirth, who have been reported to have normal upper gastrointestinal motility[11]. Differences in rectosigmoid motility have also been shown between these patient subgroups[9].

With regard to colonic transit itself, previous reports suggest that dysmotility in the acquired group is limited to the distal colon, perhaps secondary to rectal–pelvic floor dyssynergia[10,11]. Conversely, patients with chronic idiopathic symptoms are thought to suffer primarily from generalized colonic inertia[10]. However, this assumption has never been addressed. The aim of this study was to investigate whether there are differences in colonic transit between patients with STC arising *de novo* and those whose symptoms followed pelvic surgery or childbirth.

Patients and methods

Patients

The study population comprised all patients referred consecutively for scintigraphic assessment of colonic transit between 1990 and 1999. During the study period, 141 (29·8 per cent) of 473 patients undergoing investigation of intractable constipation were found to have STC (proven by radio-opaque marker study; more than 20 per cent of 50 markers administered remaining at 100 h on a plain abdominal radiograph[1,4]). Of 81 patients, whose symptoms arose either *de novo* before the age of 20 years (idiopathic STC) or followed pelvic surgery or childbirth (acquired STC), 48 went on to have colonic scintigraphy and formed the study population. The other 33 patients who met the entry criteria did not undergo scintigraphic assessment because of geographical or time constraints. The 60 remaining patients with STC fell into other subgroups, such as *de novo* adult onset or onset following a recognized central neural injury[15].

Careful history taking, clinical examination and other appropriate investigations were used to exclude secondary causes of constipation (e.g. neurological disorders, opiate abuse). Organic disease or megarectum/colon had been excluded previously by barium enema or colonoscopy. In three patients in whom the rectoanal inhibitory reflex was equivocal, a rectal biopsy had been taken which confirmed normal ganglionic morphology.

Scintigraphy

Laxative medication and opiate analgesics were stopped 24 h before the start of the study and avoided until its completion. Patients remained on their normal diet during the study period. Colonic scintigraphy was carried out as described previously[4]. Some 3·7 MBq ^{111}In-radiolabelled diethylenetriamine penta-acetic acid (Amersham International, Amersham, UK), equivalent to an effective whole-body dose of 1·1 mSv in normal individuals, was ingested orally in a small quantity of water on the afternoon of day 1 (time zero). Anterior (supine) and posterior (prone) abdominal scans were taken with a γ camera (Scintiview™; Siemens, Bracknell, UK) twice a day for the following 4 days, at 18, 24, 42, 48, 66, 72, 90 and 96 h. Any bowel movement that occurred following ingestion of the radioisotope, and before the first scan, was collected and the activity counted.

Data processing and analysis

Seven regions of interest (ROIs) were drawn around computer-generated images of the anterior and posterior abdominal scans for each patient: (1) caecum and ascending colon, (2) hepatic flexure, (3) transverse colon, (4) splenic flexure, (5) descending colon, (6) sigmoid colon and rectum, and (7) excreted faeces[4]. The percentage activity (of total ingested activity) in each ROI was calculated at each time point by customized computer software (Micas V Nucmed™ software; Park Medical Systems, Farnborough, UK), which corrected scans for decay, and averaged corresponding anterior and posterior scans to correct for tissue attenuation (geometric mean). Time–activity curves for each ROI were then generated, and also the geometric centre of isotope mass (GCI) at each time point (*Fig. 1*)[4,16]. Delineation of differential patterns of colonic transit was based on previous published guidelines: a delay throughout the entire colon (generalized delay), based on a GCI of less than 3·6 at 48 h[4,17], or a distal colonic delay only, based on a GCI of 3·6 or more at 48 h[4].

Analysis of severity of transit delay

Previous studies have given only an index of the rate of transit based upon the position of the GCI at a given time point[2–4,7], time–activity relationships within isolated colonic segments[2–4], or, in parallel with radio-opaque marker studies[1,4,7], on the amount of isotope remaining in the colon at a given time point[4]. The former methods do not give an integrated index of pancolonic transit disturbance, and the latter method is valid only in a minority of patients who pass some of the isotope during the course of the studies. Therefore, so that further comparisons regarding severity of the transit disturbance could be made, two new variables were defined: the gradient of GCI progression (gradient) and the estimated evacuation time. Both variables were reliant upon quantification of the spatial progression

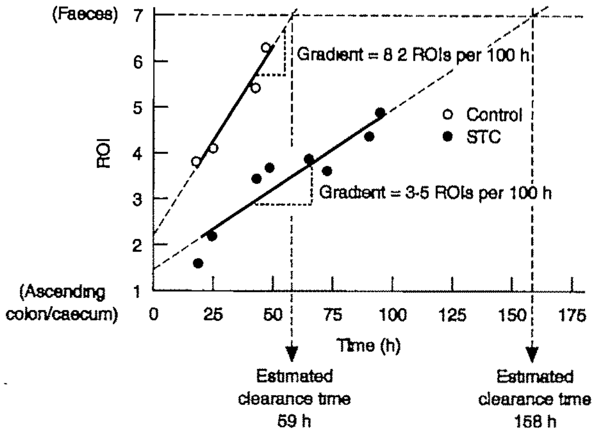


Fig. 1 Time–geometric centre of isotope mass (GCI) curves in a normal subject and in a patient with slow-transit constipation (STC). The curves have been linearized by plotting a line of best fit. The slope of each line is denoted as the gradient of GCI progression. Each line has been extrapolated and the estimated evacuation time of the isotope determined. Although there are seven regions of interest (ROIs), the calculated gradient may, when transit is normal, exceed 7 ROIs per 100 h

of GCI over the entire study period for each subject with a generalized delay. The time–GCI curve was linearized using a best-fit approach (Excel 97®; Microsoft Corporation, Redmond, Washington, USA) (*Fig. 1*), and the gradient was calculated and expressed in ROIs per 100 h. The estimated evacuation time, in hours, was calculated using $y = mx + c$, where $y = 7$ (GCI in faeces), m is the gradient, x the estimated evacuation time and c the intercept. A similar analysis was not performed for patients with left-sided delay only, because progression of the isotope throughout the study period was clearly non-linear in the majority of studies.

To give a degree of validation to these new variables, they were compared with previously defined measures of colonic transit, namely the position of GCI at 96 h[7,16,17] and the percentage retention of isotope within the colon at the conclusion of the study[4]. Variables of colonic transit were also compared with those calculated for a control group consisting of seven healthy female volunteers of median age 43 (range 22–59) years. All volunteers had a normal stool frequency (more than three per week) and no history of gastrointestinal symptoms or surgery. Use of normal control subjects was approved by the ethics committee of the London Hospital Medical College. Data from some of these subjects have been published previously[4].

Statistical analysis

Data were expressed for grouped results as median (95 per cent confidence interval (c.i.)). Comparison of test groups (patients *versus* normal subjects, and chronic idiopathic subgroup *versus* acquired subgroup) was performed using the Mann–Whitney U test for unpaired data, on the basis that the distribution of some variables showed unequal variances (Prism 2.01®; GraphPad Software, San Diego, California, USA). Linear regression analysis was used to compare scintigraphic variables with conventional measures of isotope progression, and with patient age and duration of symptoms. Contingencies were compared using Fisher's exact test. For all tests, $P < 0{\cdot}05$ was considered to show a significant difference.

Results

All 48 patients were female, with a bowel frequency of one movement or less per week. Constipation had proved resistant to normal therapeutic measures. Thirty-six patients complained of symptoms arising *de novo* (chronic idiopathic subgroup) and the remaining 12 patients had acquired their symptoms following pelvic surgery or childbirth (acquired subgroup). All patients with chronic idiopathic constipation had developed their symptoms before the age of 20 years[18] (0–5 years, $n = 18$; 6–10 years, $n = 7$; 11–15 years, $n = 6$; 16–20 years, $n = 5$). Of the 12 patients whose constipation had arisen after a pelvic event, five had acquired their symptoms following childbirth (four vaginal deliveries, one caesarean delivery) and the remaining seven following pelvic surgery (three hysterectomy, one oophorectomy, one combined hysterectomy–oophorectomy, one laparoscopic sterilization and one appendicectomy). The median age at onset in the latter group was 24 (range 16–39) years.

Patterns of colonic transit

The incidence of generalized slow-transit and left-sided colonic delay was similar between subgroups (28 of 36 patients and eight of 12 patients with generalized idiopathic and acquired STC respectively; $P = 0{\cdot}45$) (*Table 1*). The 36 patients with a generalized delay in colonic transit were considered for further analysis of transit variables.

Validation of new variables against conventional measures of isotope progression

For all subjects, the estimated evacuation time and gradient demonstrated strong linear correlations with the GCI at 96 h: $r^2 = 0{\cdot}79$ and $r^2 = 0{\cdot}77$ respectively ($P < 0{\cdot}001$ for both). In patients in whom some isotope was excreted within the study period, the estimated evacuation time and gradient demonstrated linear correlations with percentage retention of isotope at 96 h: $r^2 = 0{\cdot}88$ ($P = 0{\cdot}01$) and $r^2 = 0{\cdot}97$

Table 1 Patient data

Transit delay	No. of subjects	Age (years)	Duration of symptoms (years)	Marker retention (%)
Idiopathic				
Generalized	28	39 (16–59)	32 (3–54)*	94 (26–100)
Left	8	34 (28–62)	27 (15–47)†	80 (42–86)
Acquired				
Generalized	8	33 (21–43)	4 (2–26)	100 (22–100)
Left	4	30 (26–40)	6 (1–7)	95 (54–100)
Normal	7	43 (22–59)	—	—

Values are median (range). All patients and volunteers were women. *$P < 0\cdot001$ *versus* acquired slow-transit constipation (STC) (generalized delay); †$P = 0\cdot004$ *versus* acquired STC (left-sided delay) (Mann–Whitney U test)

($P = 0\cdot003$) respectively (the majority of patients (26 of 36, 72 per cent) retained all isotope at 96 h and were therefore excluded from this analysis).

Scintigraphic variables in slow-transit constipation (generalized delay) *versus* controls

Although there was some overlap between patients and control subjects with regard to the gradient of GCI progression (*Fig. 2a*), the difference was highly statistically significant (median 3·1 (95 per cent c.i. 2·6–3·3) *versus* 6·4 (4·1–8·8) ROIs per 100 h; $P < 0\cdot001$). The estimated evacuation time of isotope was significantly greater in the 36 patients with a generalized delay in transit than for normal volunteers, without any overlap between groups: 171 (176–231) *versus* 63 (49–74) h ($P < 0\cdot001$) (*Fig. 2b*).

Scintigraphic variables of generalized colonic delay in patient subgroups

The progression of GCI was significantly slower, and the estimated evacuation time of isotope significantly longer, in patients with chronic idiopathic STC than in patients with acquired symptoms (gradient: 2·5 (2·4–3·2) *versus* 3·7 (3·1–4·2) ROIs per 100 h, $P = 0\cdot04$; estimated evacuation time: 202 (187–251) *versus* 145 (126–171) h, $P = 0\cdot02$) (*Fig. 3*).

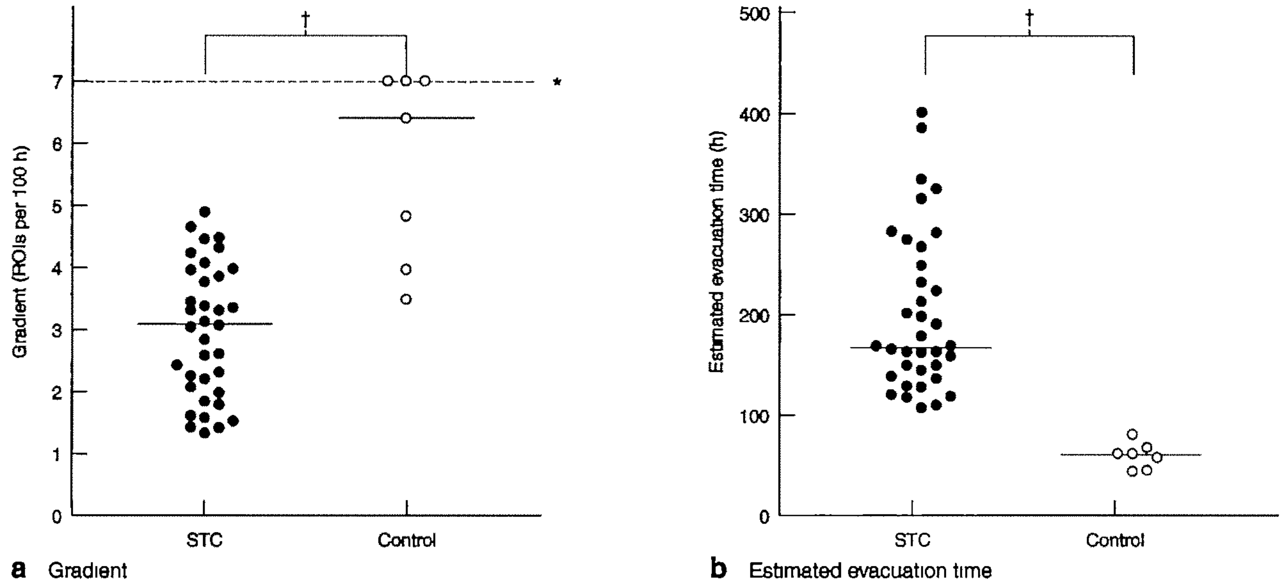
Effect of age and duration of symptoms in patients with a generalized colonic delay

Although the ages of patients in both subgroups were matched (*Table 1*), patients with chronic idiopathic STC had been symptomatic for significantly longer than patients with acquired symptoms (median 32 *versus* 4 years; $P < 0\cdot001$). Linear regression analysis showed that there was a significant correlation between gradient and estimated evacuation time with both age and duration of symptoms in patients with chronic idiopathic STC (age: $r^2 = 0\cdot20$ ($P < 0\cdot02$) and $r^2 = 0\cdot16$ ($P < 0\cdot04$); duration: $r^2 = 0\cdot30$ ($P = 0\cdot003$) and $r^2 = 0\cdot26$ ($P = 0\cdot005$) respectively) (*Fig. 4*). Conversely, no correlation was identified in the eight patients with acquired symptoms with either age ($r^2 = 0\cdot08$ ($P = 0\cdot50$) and $r^2 = 0\cdot06$ ($P = 0\cdot55$) for gradient and estimated evacuation time respectively) or duration of symptoms ($r^2 = 0\cdot13$ ($P = 0\cdot38$) and $r^2 = 0\cdot18$ ($P = 0\cdot30$) respectively).

Discussion

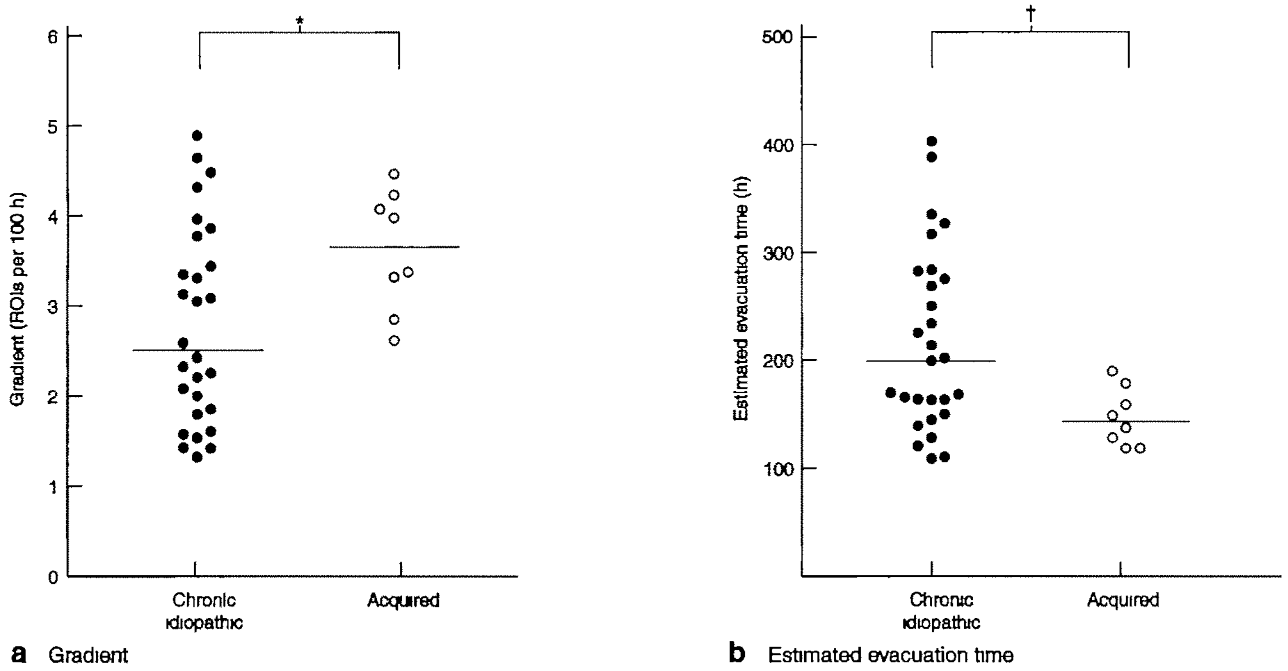
Selected patients with STC may be offered a surgical option for amelioration of their symptoms, with subtotal colectomy and ileorectal anastomosis being the operation of choice[19–21]. Although the outcome of segmental colectomy has been shown previously to be inferior[19,21], two groups have recently suggested that the outcome of limited resection, based on the findings of segmental radio-opaque marker studies, may be at least equivalent to that of total colectomy[22,23]. Knowledge of the pattern of colonic transit delay, in addition to aiding further understanding of this condition, may thus assist decision making in terms of surgical management. Whether similar information may guide future medical management is not known.

Previous radionuclide transit studies have utilized the position of the GCI at a given time point, or the retention of isotope (as a percentage of that administered)[2–4,7], as an index of the rate of transit[2–4,7]. In this study, spatial progression of the isotope was quantified for the entire study period by linearizing the time–GCI curves for each patient and calculating the slope. Such methodology relies on the application of an ordinal (ROIs being numbered 1–7), but non-interval, scale to a continuum in which the ordinates are not necessarily equal. This is a necessary simplification of the situation *in vivo*, where the real distance between each ROI, and within each ROI, is variable and cannot be estimated easily. Furthermore, intraluminal rate of flow is not constant in all areas of the colon[2,4,16,24], and fitting data to a linear function by regression analysis may therefore be too simplistic. Nevertheless, in normal subjects at least, once isotope has progressed beyond the right colon, linear regression analysis has been shown to be an appropriate model[16]. It is implicit that the calculation of estimated time of complete evacuation of isotope may be subject to the same criticism. Additionally, since this is calculated from an extrapolation of the fitted regression line, there is no guarantee that this linear relationship, evident up to 96 h, continues to its conclusion (total faecal excretion). Both variables were therefore validated against



a Gradient **b** Estimated evacuation time

Fig. 2 Scintigraphic variables in patients with slow-transit constipation (STC) and in asymptomatic control subjects. Horizontal bars denote median values. *Three volunteers had a calculated gradient exceeding 7 regions of interest (ROIs) per 100 h. †$P < 0.001$ (Mann–Whitney *U* test)

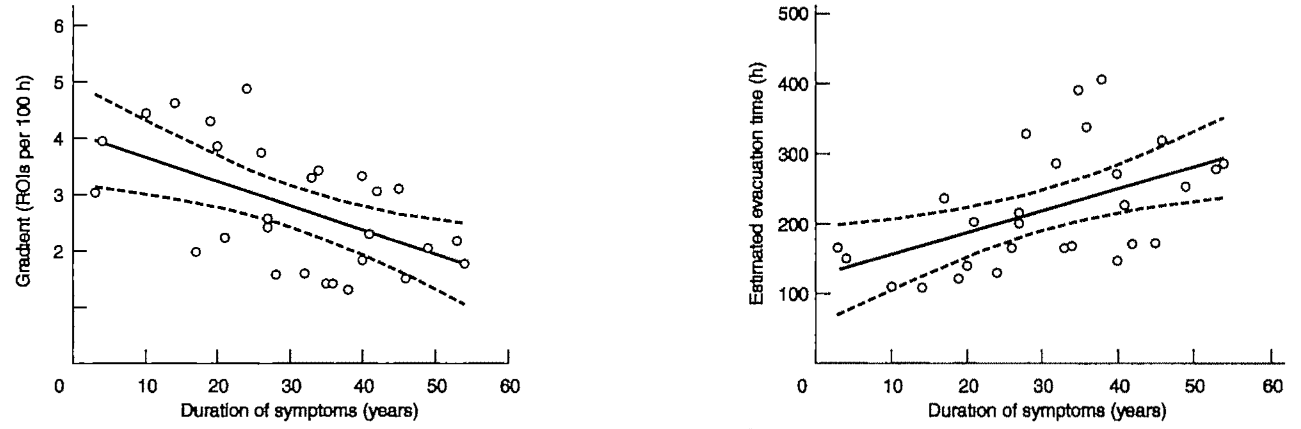


a Gradient **b** Estimated evacuation time

Fig. 3 Scintigraphic variables in patients with chronic idiopathic slow-transit constipation (STC) and in patients with acquired STC. Horizontal bars denote median values. ROI, region of interest. $^*P = 0.04$, $^\dagger P = 0.02$ (Mann–Whitney *U* test)

conventional measures of the rate of transit, and strong correlations were demonstrated.

This study is the first to have compared patterns of colonic transit in patients with chronic idiopathic or acquired STC, and has demonstrated that in fact they do not differ. Previous studies, having clearly demonstrated differential colonic transit patterns in patients with chronic constipation, have not equated findings with clinical histories[2–4,7,25]. Conversely, reports of physiological differences between patient groups with varying clinical histories



a Gradient

b Estimated evacuation time

Fig. 4 Application of linear regression modelling to examine the effect of duration of symptoms on **a** gradient and **b** estimated evacuation time in patients with chronic idiopathic slow-transit constipation and a generalized pattern of transit delay. Regression lines (solid) are shown with 95 per cent confidence intervals (broken lines). ROI, region of interest. **a** $r^2 = 0{\cdot}30$, $P = 0{\cdot}003$; **b** $r^2 = 0{\cdot}26$, $P = 0{\cdot}005$

have not considered the pattern of colonic transit itself[9,11,12]. Indeed, in studies in which patients with acquired symptoms have been investigated specifically[9,11,12], colonic scintigraphy has not been performed or has gone unreported. As in other studies in which subgroups have been compared[9,11,18], the *a priori* assignment of patients to clinical subgroups is clearly reliant upon patient recollection. Although every attempt was made to establish date and mode of symptom onset, it is possible that some patients with symptoms labelled as acquired may have had symptoms, albeit less severe, predating the pelvic event. Furthermore, to limit inclusion of patients with chronic idiopathic STC to those whose symptoms arose before the age of 20 years is somewhat arbitrary, although this cut-off value has been used previously[18].

The correlation between symptoms, including bowel frequency, and degree of transit delay, and the relationship between colonic transit and colonic contractile activity remain unclear[24,26]. However, this study has shown (using calculated variables) that, as a group, patients with generalized STC whose symptoms arose *de novo* have a more pronounced, i.e. 'severe', disturbance of transit. Previous studies have shown that the rate of transit can vary between individual patients[2–4,6,7], but the concept of severity has not been addressed. Intraindividual variation in colonic transit in healthy control subjects has been estimated at below 20 per cent, with decreasing variability over increasing transit time[27]. It is feasible that the variance in severity that has been shown in the present study may be related to the significant difference in the duration of symptoms between the two subgroups. The strong correlation between variables of transit disturbance and duration of symptoms, demonstrated for the first time by this study, is consistent with the complaint of many patients that there is a progressive nature to their condition[8,10]. There were no differences in the ages of patients in the two subgroups, but age also correlated with severity of transit disturbance in the chronic idiopathic subgroup alone. Although other aspects of anorectal function do change with age (e.g. rectal sensory thresholds and resting tone[28]), many studies in large numbers of normal volunteers, using both radio-opaque markers[29–32] and radioisotope scintigraphy[27], have failed to show increasing colonic transit times with age. Thus the independent effect of age on transit in patients with STC is unclear.

This study has demonstrated that differences in colonic transit disturbance between subgroups of patients with STC are revealed by isotope scintigraphy. The design of this study was not sufficient to conclude whether duration, a true difference in biology, or both, account for these differences. The latter hypothesis is unlikely to be resolved until further studies have shed light on the aetiology or aetiologies of STC, and have presented a consistent biological marker of both STC and its severity. The influence of duration of symptoms might be addressed by a longitudinal scintigraphic study of patients with STC.

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Original article

Blockade of growth factor receptors in ductal carcinoma *in situ* inhibits epithelial proliferation

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Background: Ductal carcinoma *in situ* (DCIS) expresses c-erbB-2 receptor and epidermal growth factor receptor (EGFR). The aim of this study was to determine whether blocking of c-erbB-2 receptor with a humanized monoclonal antibody, 4D5 (Herceptin™), or of EGFR with an epidermal growth factor receptor tyrosine kinase inhibitor (EGFR-TKI), ZD1839 (Iressa™), would decrease epithelial proliferation in DCIS.

Methods: DCIS tissue from 18 women undergoing surgery was implanted into 16 to 20 athymic nude mice per experiment (eight xenografts per mouse). Treatment commenced 2 weeks after implantation and consisted either of twice-weekly intraperitoneal injections of 4D5 10 mg/kg or of daily gavage with ZD1839 at 100–200 mg/kg for 14 days; appropriate controls were included. Xenografts were removed on days 14, 21 and 28. Proliferation was assessed by counting 1000 epithelial cells after Ki67 immunostaining.

Results: ZD1839 inhibited proliferation compared with that in controls after 14 days ($P < 0{\cdot}01$), whereas 4D5 did not.

Conclusion: Proliferation in DCIS was decreased by EGFR tyrosine kinase inhibition but not by c-erbB-2 receptor blockade. ZD1839, an orally active and selective EGFR-TKI, has potential as adjuvant therapy in DCIS.

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Introduction

The incidence of breast cancer is rising, partly as a result of earlier diagnosis by mammographic screening. Ductal carcinoma *in situ* (DCIS) is a preinvasive breast lesion, which accounts for 30 per cent of cases of screen-detected breast cancer[1]. Untreated, DCIS progresses to invasive breast cancer in 25–30 per cent of patients. Traditionally, most patients were treated by mastectomy, but this represented overtreatment for the majority of women who were not destined to develop breast cancer. However, after breast-conserving surgery, up to 40 per cent of women with DCIS develop a recurrent lesion within 15 years and, of these, half will be invasive breast cancer and therefore potentially incurable[2].

Some 70 per cent of DCIS specimens are of the comedo subtype. Comedo DCIS is associated with a high proliferative rate, high nuclear grade, negative oestrogen receptor (ER) status, epidermal growth factor receptor (EGFR) expression, and c-erbB-2 receptor overexpression[3].

The authors have previously shown *in vivo* that epithelial proliferation in high-grade comedo DCIS is unaffected by antioestrogen therapy, which suggests that some other factor(s) promote the high proliferative drive seen within this subtype of DCIS[4].

EGFR and c-erbB-2 belong to the type I family of growth factor receptors, which also includes c-erbB-3 and c-erbB-4[5,6]; EGFR and c-erbB-2 ligand stimulation result in mitogenesis[7–9]. Both EGFR and c-erbB-2 have an extracellular receptor binding domain and a cytoplasmic tyrosine kinase domain. On attachment of a ligand to these receptors, the receptors dimerize, which may result in either homodimerization or heterodimerization[8]. Dimerization results in tyrosine kinase autophosphorylation, which then activates a number of different intracellular signalling pathways, including the mitogen-activated protein kinase signalling pathway. This leads to altered gene expression, cell proliferation and prevention of apoptosis[6,10–14].

EGFR is a 170-kDa transmembrane glycoprotein that is found on many epithelial cells. Six ligands are currently known to activate EGFR: epidermal growth factor (EGF), transforming growth factor α, amphiregulin, betacellulin, epiregulin and heparin binding EGF[15]. c-erbB-2 is a 185-kDa transmembrane tyrosine kinase for which a c-erbB-2 ligand has not yet been identified. EGFR tyrosine kinases and c-erbB-2 antibody therapy have been shown to inhibit proliferation and/or induce apoptosis *in vitro* and *in vivo* in breast, ovary, lung, colonic and gastric cell lines[16–21].

The aim of this study was to determine whether inhibition of EGFR (with the orally active, selective tyrosine kinase inhibitor (TKI) ZD1839) or c-erbB-2 (with the receptor-directed monoclonal antibody 4D5) would result in decreased proliferation and increased apoptosis in DCIS.

Patients and methods

Patients

The women in this study attended either the Nightingale Breast Screening Assessment Centre or the Symptomatic Breast Clinic at the University Hospital of South Manchester, UK, between October 1997 and September 1999. Eighteen women were included in the study; all had a preoperative diagnosis of widespread DCIS with microcalcification seen on mammography. Tissue samples were obtained at therapeutic excision of DCIS. Approval to remove tissue from pathological samples in this study was granted by the South Manchester Medical Research Ethics Committee.

Mouse model

The animals used, the treatment of tissue samples, and the process of implantation and retrieval of xenografts have been described previously[4,22]; day 0 represented the day of implantation. For the ZD1839 100–200 mg/kg experiments, grafts were removed on days 14, 21 and 28. Experiments with 4D5 and ZD1839 75 mg/kg involved grafts being removed on days 14, 28 and 42.

In the first set of the experiments, mice received intraperitoneal injection of either 4D5, a humanized monoclonal antibody directed against an epitope on the extracellular domain of c-erbB-2 (Herceptin™; Genentech Pharmaceuticals, San Francisco, California, USA), or control antibody DA4-4 (Genentech Pharmaceuticals). DA4-4 is a non-specific human immunoglobulin (Ig) G of the same class and isotype as 4D5. The dose of 4D5 and DA4-4 administered was 10 mg/kg; a similar concentration has been shown to have an antitumour effect in similar *in vivo* models using this antibody, and is equivalent to the dose used in humans[23,24].

In the second set of experiments the mice were gavaged with either ZD1839, an orally active, selective EGFR-TKI (donated by AstraZeneca Pharmaceuticals, Alderley Park, UK), or vehicle control (0·5 per cent polysorbate).

Positive control experiments

The human breast carcinoma cell line MDAMB231 overexpresses EGFR, whereas the human ovarian carcinoma cell line SKOV3 overexpress c-erbB-2, so these cell lines were used as the positive controls for the ZD1839 and 4D5 experiments respectively. Both cell lines were obtained from the American Type Culture Collection (Rockville, Maryland, USA) and grown in Dulbecco's modified Eagle's medium supplemented with 10 per cent fetal calf serum (FCS) (Sigma, Poole, UK), insulin, sodium pyruvate and hydrocortisone (Sigma). Two million MDAMB231 cells in a 200-µl solution of 10 per cent FCS with 50 per cent (v/v) Matrigel (Collaborative Biomedical, Bedford, UK) were injected subcutaneously into each flank (two tumours per mouse) of each mouse ($n = 15$), allowed to grow for 3 weeks, and then gavaged daily with either ZD1839 200 mg/kg or vehicle for 14 days. The SKOV3 injections were administered in the same way. The tumours were allowed to grow in the mice ($n = 20$) for 14 days; the animals were then given intraperitoneal injections of either 4D5 10 mg/kg or control antibody every 72 h. Tumour diameters were measured with calipers twice weekly, and tumour volume was calculated from the formula[25]: tumour volume = $(\text{width})^2 \times \text{length}/2$.

Immunohistochemistry

Tissue sections taken from the xenografts were fixed with formalin and embedded in paraffin. Antibody against EGFR (NCL-EGFR; Novocastra, Newcastle upon Tyne, UK), c-erbB-2 (MAB4022; Chemicon, Harrow, UK), ER (M7047; Dako, High Wycombe, UK) and Ki67 (MIB-1; Coulter, UK) was used to detect protein expression. After incubation with the primary antibody, an appropriate biotinylated IgG was applied to the tissue sections, followed by incubation with the avidin biotinylated complex reagents (K0377; Dako). The staining was developed in the diaminobenzidine chromagen substrate solution. Mayer's haematoylin was used as a counterstain.

Assessment of apoptosis, proliferation, oestrogen receptor, c-erbB-2 and epidermal growth factor receptor

The scoring methodologies for apoptosis, proliferation and ER have been described in detail previously[22]. Epithelial proliferation was expressed as the Ki67 labelling index (LI) and apoptosis as the apoptotic index (AI). The indices were obtained by counting at least 1000 DCIS epithelial cells and expressing the percentage of positive cells. EGFR and c-erbB-2 were assessed semiquantitatively.

Statistical analysis

For each DCIS specimen, a comparison was made between DCIS samples retrieved from 4D5- and ZD1839-treated and control mice at each assessment timepoint. The tissue samples obtained at each timepoint in the two study groups were considered to be statistically independent and were compared with the Mann–Whitney *U* test as the distributions in the groups were non-parametric. All significance tests were two sided, using the conventional 5 per cent significance level. The Mann–Whitney *U* test was also applied to differences in tumour growth in the MDAMB231 and SKOV3 experiments.

Results

Breast tissue from 18 women was used in this study. The median age was 49 (range 34–74) years. Three patients underwent wide local excision (two with axillary node clearance) and 15 had a mastectomy (nine with axillary clearance). On histological examination, seven women were diagnosed as having only DCIS, and 11 had invasive carcinoma with DCIS. Comedo-type DCIS was present in 14 of the 18 specimens, of which ten were of high nuclear grade and four of intermediate nuclear grade. Of the four specimens containing non-comedo DCIS, there was one each of low and intermediate nuclear grade, and two of high nuclear grade.

Xenograft implant and retrieval

Non-implanted tissue pieces (day 0 specimens) were examined to determine the proportion of the xenografts expected to contain DCIS. For example, in an experiment in which 20 per cent of day 0 specimens contained DCIS, one in five retrieved xenografts would be expected to return DCIS. However, if only one in ten retrieved xenografts returned DCIS, a 50 per cent return was recorded. A total of 367 day 0 samples was obtained, of which 91 (24·8 per cent) contained foci of DCIS (median number of day 0 specimens per experiment containing DCIS 17·6 (range 5·2–35·0) per cent). Some 3232 xenografts were implanted, of which 3150 (97·5 per cent) of were retrieved. Of these 3150 xenografts, 406 (12·9 per cent) contained DCIS; the rest contained stroma and/or normal breast epithelium. The median percentage of retrieved xenografts containing DCIS per experiment was 13·7 per cent; therefore the median retrieval of DCIS per experiment was 78·2 per cent of that expected. All xenografts retained good histological morphology and were reviewed by a consultant breast histopathologist.

Epithelial proliferation and apoptosis in ductal carcinoma *in situ*

SKOV3-positive control experiment

There was a significant inhibition of median (interquartile range (i.q.r.)) SKOV3 tumour volume growth after 14 days' treatment (day 35) with 4D5 compared with controls (58·8 (29·7–116·9) *versus* 229·3 (114·0–324·1) mm^3, $P < 0·01$) (*Fig. 1*).

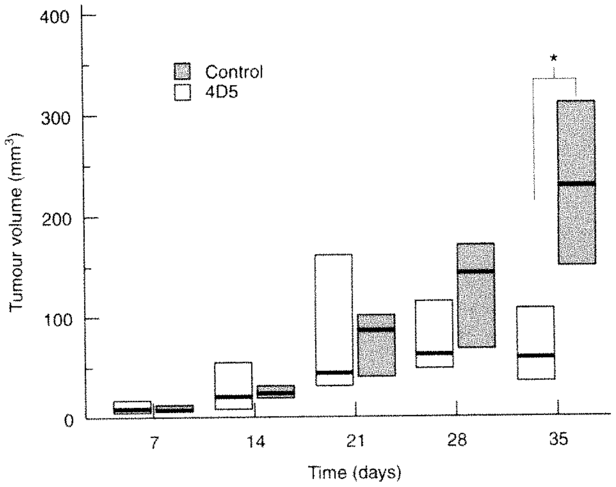
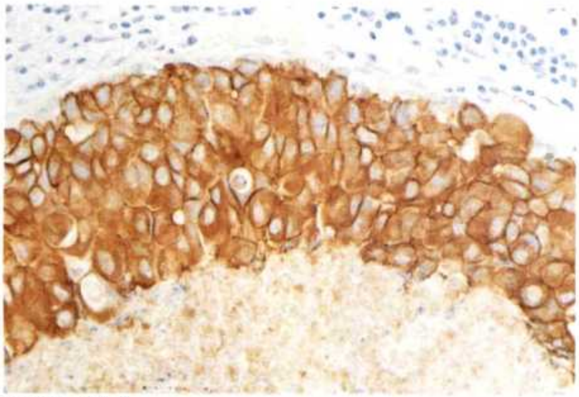
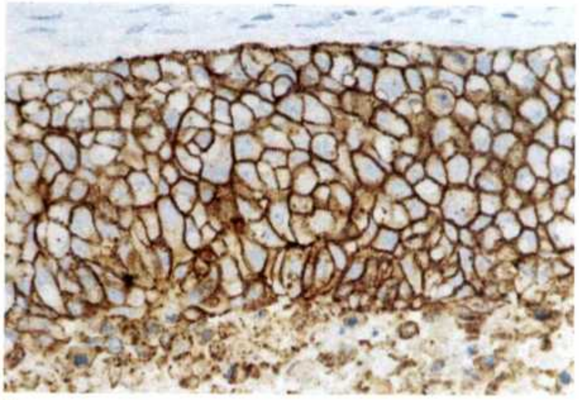


Fig. 1 Effect of the c-erbB-2 monoclonal antibody (4D5) on SKOV3 tumour growth *in vivo*. Horizontal lines represent median values; boxes denote the interquartile range. Significant growth inhibition occurred in the 4D5 group after 3 weeks of treatment (day 35): $^*P < 0·01$ (4D5 *versus* control, Mann–Whitney *U* test)



a C-erbB-2



b EGRF

Fig. 2 Photomicrographs of sections of ductal carcinoma *in situ* (DCIS) stained immunohistochemically for **a** c-erbB-2 membrane receptor and **b** epidermal growth factor receptor (EGRF) (original magnification × 400)

C-erbB-2-positive and -negative ductal carcinoma in situ

Of seven DCIS xenograft experiments in which 4D5 was used, five were immunohistochemically c-erbB-2 positive (*Fig. 2a*). The median semiquantitative score for the c-erbB-2-positive DCIS was 3+ (range 1–4). All seven were comedo-type DCIS and ER negative. Three were of high nuclear grade and four of intermediate nuclear grade.

Median (i.q.r.) epithelial proliferation (LI) and apoptosis (AI) for the day 0 c-erbB-2-positive DCIS were 16·8 percent (11·7–23·4) and 0·65 percent (0·53–0·88) respectively. There was no significant decrease in LI or AI on day 14. No differences in median (i.q.r.) LI or AI were noted on comparison of the 4D5-treated group with the control group on day 28 (LI 9·8 (6·6–19·1) *versus* 16·2 (9·0–26·3), $P = 0{\cdot}21$; AI 0·47 (0·18–1·19) *versus* 0·37 (0·23–0·69), $P = 0{\cdot}79$) or day 42 (LI 24·5 (11·6–36·8) *versus* 19·9 (15·0–35·2), $P = 0{\cdot}88$; AI 0·72 (40–1·07) *versus* 0·96 (0·83–1·77), $P = 0{\cdot}14$). Similar non-significant results were seen with the c-erbB-2-negative 4D5-treated *versus* control DCIS xenografts.

MDA MB231-positive control experiment

There was inhibition of the median (i.q.r.) MDA MB231 tumour volume growth after 7 days of treatment (day 28) with ZD1839 compared with controls (61·8 (36·5–78·8) *versus* 107·0 (85·3–161·1) mm^3, $P < 0{\cdot}01$) (*Fig. 3*).

Epidermal growth factor receptor-positive ductal carcinoma in situ

Ten of the 11 DCIS xenograft experiments in which ZD1839 was used were immunohistochemically EGFR positive (*Fig. 2b*). The median semiquantitative score for EGFR-positive DCIS was 2+ (range 1–3). Two of the ten EGFR-positive DCIS specimens were also ER positive.

There was no significant change in either LI or AI from day 0 to day 14. However, after 14 days of treatment (day 28) with ZD1839 100–200 mg/kg there was a significant decrease in LI compared with the value in controls (*Fig. 4*) and this effect was maintained when treatment was extended to 28 days (day 42) at ZD1839 75 mg/kg ($P < 0{\cdot}05$; data not shown). After 7 days of treatment (day 21) there was a significant increase in median (i.q.r.) AI in the ZD1839-treated group compared with controls (2·1 (1·1–2·3) *versus* 0·6 (0·5–1·2), $P < 0{\cdot}01$); however, after 14 days (day 28) of ZD1839 the median (i.q.r.) AI was still higher than that in controls, although this was not significant (0·81 (0·40–1·62) *versus* 0·40 (0·36–1·21), $P = 0{\cdot}41$). There was no significant difference in LI or AI when comedo-type DCIS was compared with non-comedo DCIS treated with ZD1839 (data not shown).

Epidermal growth factor receptor-negative ductal carcinoma in situ

There were no significant differences in LI or AI between the two groups at all timepoints assessed.

Mouse weight

Toxicity was assessed by regular monitoring of the weight and general well-being of the mice. There was no significant difference in the median (i.q.r.) weight of mice treated with ZD1839 100 mg/kg (23·4 (20·0–28·0) g) or 4D5 (24·2 (22·1–25·5) g) compared with controls (23·8 (23·4–26·1) g). No toxic effects were seen at these doses.

Discussion

This study has shown that EGFR tyrosine kinase inhibition with an orally active and selective inhibitor ZD1839

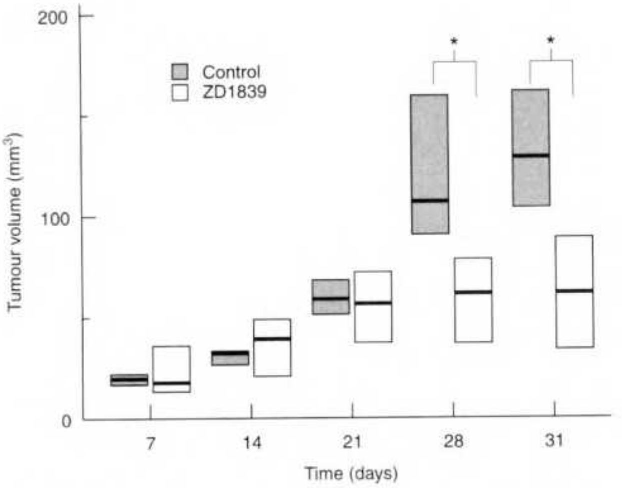


Fig. 3 Effect of the epidermal growth factor receptor tyrosine kinase inhibitor ZD1839 on MDA MB231 tumour growth *in vivo*. Horizontal lines represent median values; boxes denote the interquartile range. Significant growth inhibition occurred in the ZD1839 group after 2 weeks' treatment (days 28 and 31): $^*P < 0{\cdot}01$ (ZD1839 *versus* control, Mann–Whitney U test)

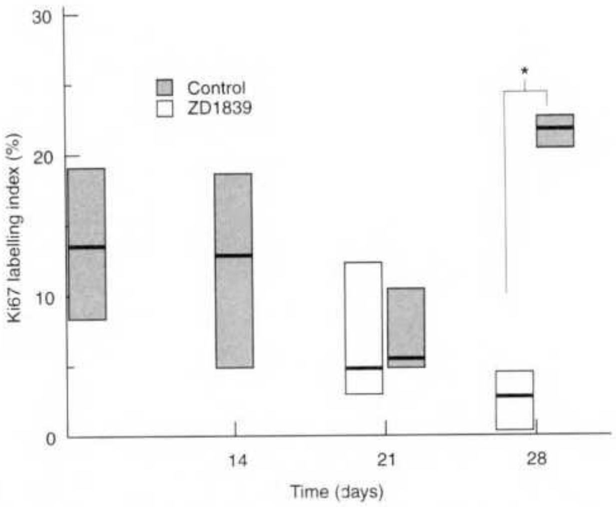


Fig. 4 Effect of ZD1839 (200 mg/kg daily) on the proliferation (Ki67 labelling index) of ductal carcinoma *in situ* epithelium in retrieved xenografts. Horizontal lines represent median values; boxes denote the interquartile range. The number of xenografts was 12, 18, seven and seven at days 0, 14, 21 and 28 respectively in the control group, and seven and ten at days 21 and 28 in the ZD1839 group. A significant decrease in proliferation was seen in the ZD1839 group after 14 days' treatment (day 28): $^*P < 0{\cdot}01$ (ZD1839 *versus* control, Mann–Whitney U test)

decreases proliferation and increases apoptosis in EGFR-positive DCIS. The c-erbB-2 monoclonal antibody 4D5 showed no inhibition in either c-erbB-2-positive or -negative DCIS.

Phase III studies with 4D5 (Herceptin$^{\text{TM}}$) have shown clear benefit in a proportion of women with metastatic c-erbB-2-overexpressing breast cancer which had disease progression despite standard chemotherapy regimens.

The lack of response noted in this series of experiments with 4D5 may be due to a number of potential reasons. By definition, DCIS has an intact basement membrane which has not been breached by the malignant cells. This intact membrane may itself present a physical barrier to the c-erbB-2 antibody, precluding interaction of the protein with the c-erbB-2 receptor. The problem of substance permeation into ducts containing DCIS has been documented previously by Holland *et al.*[4], who noted that tritiated thymidine penetrated only into the periphery and rarely into the central area of ducts containing DCIS. The absence of basement membrane around the SKOV3 xenografts would have allowed the 4D5 antibody direct access to the extracellular domain of c-erbB-2 receptor in the c-erbB-2-positive control experiment, to give rise to the cytostatic inhibitory growth effect seen.

Tyrosine kinase inhibitors are much smaller molecules (molecular weight less than 1000 kDa) than antibodies. ZD1839 has a molecular weight of 446 kDa. The smaller size theoretically gives this molecule a greater chance of penetrating through the basement membrane of DCIS.

ZD1839 is 100 times more specific for EGFR tyrosine kinase than c-erbB-2 tyrosine kinase, and 1000 times more specific for EGFR tyrosine kinase than c-flt-1 tyrosine kinase[26]. *In vitro* and *in vivo* inhibition of proliferation has been reported[27] in a wide range of EGFR-expressing cell lines treated with ZD1839. Phase I/II studies of ZD1839 have shown tumour response in a wide range of advanced tumours (including breast) with generally mild and reversible toxicity[28].

The effectiveness of novel cancer treatments targeted to EGFR function hinges on whether proliferation and survival of the treated tumour is dependent on EGFR-mediated signals. The authors have previously reported that proliferation is not driven by oestrogen stimulation in high-grade DCIS[4]. The inverse relationship of EGFR with the ER is well established[29]. *In vitro* studies with tamoxifen-resistant MCF7, a malignant breast cell line, showed a significantly marked decrease in proliferation when treated with ZD1839, suggesting that proliferation is driven mainly via EGFR signalling in these cells[30].

The suppression of apoptosis is a fundamental characteristic of cancer cells. Therefore, the demonstration of an increase in apoptosis suggests a potential for EGFR blockade by ZD1839 in the treatment of DCIS[31,32]. The mechanism by which EGFR-TKIs activate apoptosis remains unclear. Apoptosis induced by PD153035 (a different EGFR-TKI) has been shown to be associated with a marked time-dependent increase in caspase 3-like activity as well as proteolysis of poly ADP-ribose polymerase-1[33].

Dimerization is a prerequisite for tyrosine kinase activation. c-erbB-2 is the preferred partner for the other type 1 tyrosine kinase receptors (EGFR, c-erbB-3 and c-erbB-4)[34]. The frequency of EGFR heterodimerization with c-erbB-2 is not known, but it can be hypothesized that, if this is the main heterodimer formed, an EGFR-TKI will inhibit the signalling from both the EGFR and c-erbB-2, resulting in decreased proliferation and increased apoptosis.

In conclusion, these findings suggest that EGFR-TKIs have dual action on proliferation and apoptosis in EGFR-positive DCIS. They might be effective for treatment of DCIS composed of such cells. These observations encourage additional preclinical and clinical testing of EGFR inhibitors as potential agents for the treatment and chemoprevention of DCIS.

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Original article

Clinical significance of mutator phenotype and chromosome 17p and 18q allelic loss in gastric cancer

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Background: Tumour stage is the only reliable prognostic factor for gastric cancer. The molecular anomalies involved in this process have the potential to serve as additional prognostic markers.

Methods: Forty-four gastric cancers, treated by surgery alone, have been analysed for chromosome 17p and 18q allelic loss and for the presence of microsatellite instability (MSI), using microsatellite markers and DNA from paraffin-embedded tumours.

Results: Eight cancers showed a MSI-positive (MSI+) phenotype. Among the 36 MSI-negative cancers, chromosome 17p and 18q allelic losses were found in 22 of 34 and 19 of 33 informative cases respectively. Multivariate survival analysis indicated MSI status to be an independent prognostic factor along with the tumour stage. MSI+ cancers were associated with longer patient survival, whereas MSI-negative cancers had a significantly poorer prognosis ($P = 0.007$), with a median actuarial survival of 24 months.

Conclusion: MSI status is an independent prognostic factor among gastric cancers at the same stage. Chromosome 17p and 18q status added no additional prognostic information to that of tumour stage. The combined use of tumour stage and MSI status may help in deciding whether patients with advanced gastric cancer require additional therapy other than surgery alone.

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Introduction

Carcinogenesis and progression of malignancy is a multistep process resulting from the accumulation of alterations in genes involved in the control of cell proliferation and death. Recent data suggest that two molecular pathways are involved in the pathogenesis of sporadic gastric cancer[1].

The large majority of cancers are characterized by gross chromosomal changes and by allelic losses (loss of heterozygosity, LOH) in an average of at least 25 per cent of randomly chosen DNA sequences[2,3]. Common regions of LOH in a given cancer identify the chromosomal regions that contain tumour suppressor genes whose inactivation might play an important role in the initiation or progression of that cancer type. A recent allelotype study of xenografted cancers has shown that LOH at chromosome arms 17p13 and 18q21, at sites including or closely linked to *p53* and *DCC/DPC4* (deleted in colon cancer/deleted in pancreatic cancer) tumour suppressor genes, are among the most frequent allelic imbalances detected in advanced gastric cancers[3]. These chromosomal losses have been associated variably with different stages of tumour progression[2–5], while one study reported that chromosome 18q status has prognostic significance in gastric cancer[6].

A subset of sporadic gastric cancers shows widespread somatic alterations in the length of simple, repetitive genomic sequences of 1–4 base pairs (bp) (microsatellites), as the result of defective DNA mismatch repair genes (mutator genes)[7]. These cancers rarely show chromosomal losses and have been described by various terms, including replication error (RER)-positive (RER+) phenotype, ubiquitous somatic mutation phenotype and microsatellite mutator phenotype[1,8]. Conflicting results have been reported concerning the frequency of RER+ (9–45 per cent) in gastric cancer and the association of this phenotype with various clinicopathological characteristics, including survival[8–17]. In a recently held conference of the National Cancer Institute (USA), certain criteria for RER determination were proposed, with the distinction of cancers showing high or low levels of microsatellite instability (MSI-H and MSI-

L respectively) and the conclusion that these criteria could be applied only to colorectal cancer[18]. However, subsequent work suggests that similar criteria could also apply to sporadic gastric cancers[7,19–21].

In the present study, 44 sporadic advanced gastric cancers have been analysed for the occurrence of MSI and for LOH at chromosomes 17p and 18q in order to evaluate the clinical significance of these molecular markers.

Materials and methods

Selection of cases

Forty-four of 82 patients affected by sporadic gastric cancer who underwent surgery between February 1991 and June 1994 were selected for study. Criteria for selection were (1) histological diagnosis of advanced gastric cancer; (2) radical surgical removal with resection margins free from microscopic disease; (3) treatment by surgery alone with no preoperative or postoperative chemotherapy or radiotherapy; and (4) no family history of gastric or other cancers. Thirty-eight patients were excluded from the study owing to death within the first month of surgery (four patients), non-radical surgery (14 patients) or a diagnosis of early gastric cancer (20 patients).

Patients, tumours and tissue microdissection

The clinicopathological characteristics of the 44 patients are summarized in *Table 1*. Histological classification was according to Laurén[22] and the unified 1987 tumour node metastasis system for gastric carcinoma was used for pathological staging[23]. Personal and family histories were obtained by direct interview of patients and/or their relatives and family physicians. None of the 44 patients included in the study had a family history suggestive of hereditary non-polyposis colorectal cancer. Normal and tumour DNA was extracted from microdissected paraffin-embedded tissues[24].

Microsatellite analysis, definition of replication error-positive tumours and allelic loss analysis

Microsatellites were examined by polymerase chain reaction (PCR) amplification on nine loci from five different chromosomes. These included BAT-26 and BAT-40 (poly(A) mononucleotide repeats located in introns of the *hMSH2* gene and 3-β-hydroxysteroid dehydrogenase genes respectively)[25]; D1S158, a CT$_{24}$ dinucleotide repeat localized at chromosome 1q32–q41; three CA$_{(n)}$ dinucleotide repeats were D17S559, D17S1176 and D17S513 on chromosome 17p13; and three CA$_{(n)}$ dinucleotide repeats were D18S61, D18S55 and D18S58 on chromosome 18q21. PCR analysis was performed and evaluated as described previously[24,26]. A tumour was defined as RER+ when MSI was found in more than 50 per cent of the loci analysed. The presence of LOH was defined by the loss of intensity of one allele in the tumour sample with respect to the matched allele from normal tissue with a relative intensity of the two alleles in the tumour DNA differing from the relative intensity in the non-neoplastic tissue DNA by a factor of at least two.

Table 1 Clinicopathological data of 44 patients with gastric cancers

	No. of patients
Sex ratio (M : F)	29 : 15
Mean(s.d.) age (years)	65·7(10·6)
Site	
Fundus	14
Corpus	10
Antrum	19
Linitis	1
Histology	
Intestinal	28
Diffuse	8
Mixed	8
Grade	
Well differentiated	1
Moderately differentiated	23
Poorly differentiated	20
TNM stage	
IB	5
II	5
IIIA	15
IIIB	16
IV	3

TNM, tumour node metastasis

Analysis of target genes in repication error-positive tumours

Detection of mutations in receptor type II for tumour growth factor beta (*TGFβ-RII*) and *BAX*, the two genes most frequently mutated in RER+ cancers, was performed on the eight RER+ tumours, the seven tumours showing rare allelic shifts, and seven tumours selected randomly among the remaining 22 RER-negative tumours. The *TGFβ-RII* gene was analysed by PCR amplification of nucleotides 665–737, which contains the mutationally favoured 10-bp polyadenine tract (codons 125–128)[27]. Alterations in the polyadenine tract of the *TGFβ-RII* gene were detected as described previously[28]. The 94-bp region encompassing the poly-G tract of the *BAX* gene was amplified using conditions described elsewhere[29].

Follow-up and statistical analysis

The follow-up programme consisted of a minimum 5-year observation for patients who did not die from the disease. No patient was lost to follow-up, which was based on clinical, laboratory and echographic examination of the patients at 4-month intervals for the first 2 years, 6-month intervals for the next 3 years and then yearly intervals. In all patients who died from the disease, the relapse was ascertained by radiology or echography, fine-needle aspiration biopsy and in selected cases by laparotomy and biopsy. The primary statistical outcome was overall survival measured from the date of surgery. Death from cancer was the endpoint; one cerebrovascular death was treated by censoring. The overall survival distribution was calculated by the product-limit method and analysed using the Mantel–Cox test. Multivariate survival analysis was performed using Cox's proportional hazard model. Two different survival analyses were performed. The first included all potential prognostic factors (age, sex, site, size, histological type, grade, stage) and RER status. In the second model, status of chromosomes 17p and 18q was included and patients whose tumour had widespread MSI were excluded, owing to the inherent difficulty of assessing LOH in these tumours. To select the more parsimonious model, a backward elimination procedure was used including all conventional factors (age, sex, size, grade, stage and nodal metastases), RER status, and chromosome 17p and 18q status. The final model included only those factors that consistently retained significance ($P < 0.05$). The BMDP (BMDP, Los Angeles, California, USA) and SPSS® (SPSS, Chicago, Illinois) statistical programs were used.

Results

Microsatellite instability analysis

All tumours were successfully amplified by PCR for at least six of the markers used. Eight cancers showed instability in at least three of the dinucleotide repeats and were classified as RER+ cancers (*Fig. 1*). Each of these also showed instability in BAT-40 and/or BAT-26. Six of the eight RER+ cancers showed *TGFβ-RII* and/or *BAX* mutations, consisting of a 1-bp deletion in most cases and a 1-bp insertion in two cases shown in *Fig. 1*. In particular, four tumours had a *TGFβ-RII* mutation, one had a *BAX* mutation and the last showed mutations in both genes.

Seven additional tumours showed allelic shifts extending or shortening the normal allele size by 2 bp, involving one or two single alleles per case; thus in this subset, a mean 2 per cent of single alleles per case had a minimal shift. These were considered as RER-negative cancers and none showed abnormalities in BAT-26, BAT-40, *TGFβ-RII* or *BAX* mononucleotide sequences.

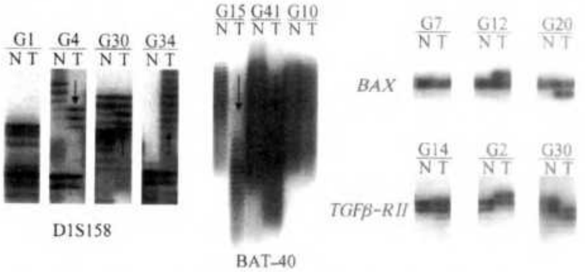

Fig. 1 Selected examples of microsatellite instability. The markers used are as indicated. N, normal; T, tumour. Expansion (upward arrows) or contraction (downward arrows) of the microsatellite sequences indicating instability is seen in cases G4, G30 and G34 with D1S158, and cases G15 and G41 with BAT-40. *BAX* mutations are also evident in cases G12 and G20 as well as *TGFβ-RII* alterations in cases G2 and G30

Allelic losses at chromosomes 17p13 and 18q21

The choice of markers also allowed the assessment of LOH on chromosomes 17p and 18q in the 36 RER-negative cancers; such losses were detected in 22 of 34 and 19 of 33 informative cases, respectively. Eight cancers harboured chromosome 17p LOH alone and five showed 18q LOH alone. LOH on both chromosomes were found in 13 cases and appeared to parallel the progression of cancer stage and unfavourable prognosis. In fact, coexistent losses were found in only one of six informative stage I or II and in 12 of 25 informative advanced stage cancers. All but one patient whose cancer showed coexistent chromosome 17p and 18q LOH died from the disease, while four of the eight patients whose cancer harboured chromosome 17p LOH alone and two of the five with 18q LOH alone were among long-term survivors.

Clinicopathological features

Seventeen of the 44 patients were alive after a period ranging from 62 to 130 months, including eight of the ten patients whose cancer was at stage I–II, six of 15 with stage IIIA disease and three of 19 with stage IIIB–IV lesions. One patient with stage I cancer died from brain infarction. Seven of the eight patients with RER+ neoplasms were among long-term survivors. Among the 26 patients who died from the disease, 18 died within 2 years after surgery and only three survived more than 5 years. Interestingly one of these latter three patients had the mutator phenotype.

Survival analysis

Multivariate analysis was carried out including all conventional factors (age, sex, site, size, histological type, grade, stage) and RER status. Only tumour stage and RER status were selected as independent prognostic factors (*Table 2*). The second multivariate survival analysis, including chromosome 17p and 18q status and excluding RER status, selected only stage as a prognostic factor (*Table 3*). The prognostic value of stage and RER status was then assessed by univariate analysis using the product-limit survival method, which showed a significantly poorer survival for patients with advanced stage ($P = 0{\cdot}005$, Mantel–Cox test; *Fig. 2*) and with RER-negative cancers ($P = 0{\cdot}007$, Mantel–Cox test; *Fig. 3*). As evident from *Figs 2* and *3*, both RER status and tumour stage showed a proportional risk in the product-limit survival analysis. The median(s.e.) actuarial survival time was 24(12) (95 per cent confidence interval (c.i.) 0–48) months for patients with RER-negative tumours whereas it was not measurable for RER+ cases, as seven of eight patients were still alive. The median actuarial survival time was 24(13) (95 per cent c.i. 0–50) months for patients with stage III–IV tumours whereas it was not measurable for stage I–II cases, as eight of ten patients were still alive. Among the 34 patients with stage III–IV disease only one of the five patients with RER+ cancer died after 68 months of follow-up, while 24 of the 29 with RER-negative tumours died with a median survival time of 19 months ($P < 0{\cdot}0008$).

Table 2 Multivariate statistical analysis (Cox's model) including replication error status

Variable	No. of survivors	No. of deaths	Relative risk of death*	*P*
Stage				
I–II	8	2	1	
III–IV	9	25	6·0 (1·4–25·7)	0·015
RER				
Positive	7	1	1	
Negative	10	26	9·2 (1·2–68·6)	0·029

Values in parentheses are 95 per cent confidence intervals. *Hazard ratio adjusted for age, sex, site, size, histological type and grade. RER, replication error

Table 3 Multivariate statistical analysis (Cox's model) including chromosome 17p and 18q status

Variable	No. of survivors	No. of deaths	Relative risk of death*	*P*
Stage				
I–II	8	2	1	
III–IV	9	25	8·3 (1·1–62·3)	0·039

Values in parentheses are 95 per cent confidence intervals. *Hazard ratio adjusted for age, sex, site, size, histological type, grade, and chromosome 17p and 18q status

Discussion

The present panel of cancers was derived solely from patients with advanced gastric cancer who underwent potentially curative resection in a single centre and forms part of a rigorous follow-up programme. These patients represented 54 per cent (44 of 82) of the total number of gastric cancers observed in the period considered. In this set of cancers: (1) eight showed the RER+ phenotype as defined by the instability in at least 50 per cent of dinucleotide markers; (2) all RER+ cases showed instability in BAT-40 and/or BAT-26; (3) six of the eight RER+ cancers showed

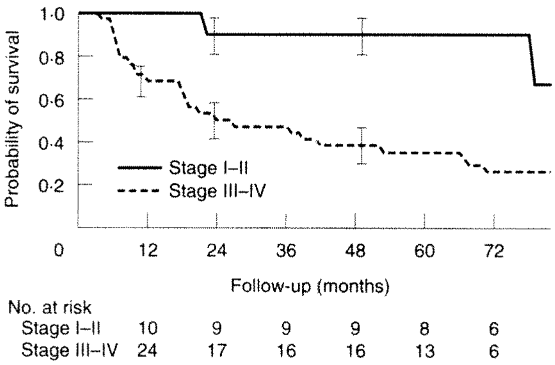

Fig. 2 Survival curve (with 95 per cent confidence intervals) obtained by the product-limit method for patients with stage III–IV cancers significantly differs from that for patients with stage I–II cancers ($P = 0{\cdot}005$, Mantel–Cox test)

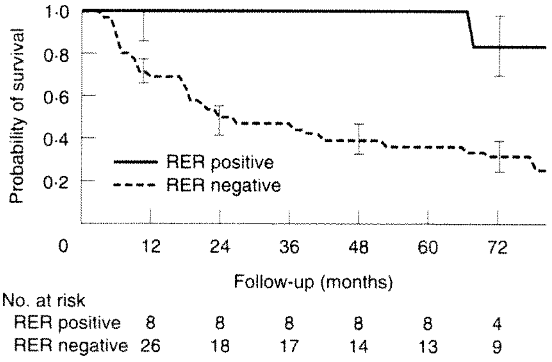

Fig. 3 Survival curve (with 95 per cent confidence intervals) for patients with replication error (RER)-positive cancers differs significantly from that for patients with RER-negative cancers ($P = 0{\cdot}007$, Mantel–Cox test)

frameshift mutations in *TGFβ-RII* and/or *BAX* genes; (4) the RER+ phenotype was a favourable and independent prognostic factor by multivariate survival analysis; and (5) the remaining 36 cancers showed a high frequency of chromosome 17p and 18q LOH which were not of prognostic value.

Only cancers exhibiting instability in at least half of the markers were defined as RER+. Using these criteria, eight of 44 cancers were classified as RER+ and all of them also showed instability in BAT-26. The variable reported frequency (9–45 per cent) of the RER+ phenotype in gastric cancer and consequential inconsistent association of this phenotype with different clinicopathological characteristics, including survival, can most likely be explained by the fact that different criteria have been used, i.e. the number and type of microsatellites analysed and how many were unstable[8–17]. At a recently held workshop, a panel of two mononucleotide and three dinucleotide repeat microsatellites was proposed for RER determination[18]. Cancers showing instability in at least two markers were defined as MSI-H while those with only one unstable microsatellite had low instability (MSI-L). Furthermore, it was concluded that these criteria could be applied only to colorectal neoplasms and that cancers of the MSI-L category shared similar clinicopathological characteristics, including a more aggressive clinical course, with MSI-negative cancers. More recent work, however, suggests that stricter criteria should be used, that is RER+ cancers should show instability in at least 50 per cent of loci when analysing six or more dinucleotide microsatellites[7,13,19–21,26,28,30,31]. Alternatively, instability in one mononucleotide repeat may be diagnostic of RER+[7,19–21,31]. None the less, using either of the last two criteria, RER+ gastric cancers show distinct clinicopathological characteristics and a different set of altered genes with respect to RER-negative cancers[19–21,30,31]. In this regard, alterations in *TGFβ-RII* and *BAX* genes are a common feature of RER+ cancers and were also found in the majority of the present RER+ cancers.

Seven cancers in this series showed rare allelic shifts, i.e. at only one or two dinucleotide microsatellite loci. They were considered to be RER-negative and did not have either *TGFβ-RII* or *BAX* mutations. All these patients died from the disease and survival analysis showed no difference between these patients and their RER-negative counterparts. The occurrence of instability at few microsatellite loci may represent a random event. In this case, it is likely that the frequency of allelic shifts in common (RER-negative) cancers might be one of the measures of the overall genomic instability, by establishing the background rate of replication infidelity of simple repeats in that particular type of cancer. For example, in this panel of gastric cancers the calculated average frequency of instability in a single allele was 2 per cent, a figure similar to that found in colorectal and duodenal cancers[26,32], but higher than the 0·3–0·8 per cent observed in pancreatic cancers[33].

Among the more common RER-negative gastric cancers, LOH on chromosomes 17p and 18q are common events[2–5] and have been variably associated with various stages of tumour progression. One recent report found chromosome 18q status to be a prognostic indicator in gastric cancer[6]. The data on microdissected samples of primary cancers confirm that the high frequency of chromosome 17p and 18q losses overlaps that found in xenografted tumours[3]. Although neither anomaly was useful for prognosis in this

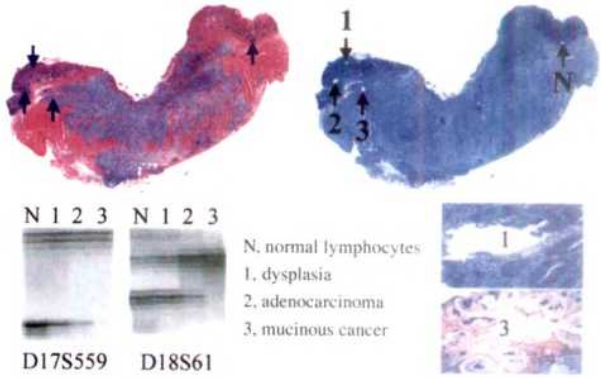

Fig. 4 Determination of allelic loss demonstrating heterogeneity and clonal expansion in the progression of a malignancy. Two consecutive paraffin sections of the cancer are shown at the top before (left) and after (right) microdissection of the areas indicated. The whole mount section (4·5 × 2·5 cm) stained with haematoxylin and eosin (left) served as guide to identify the areas to microdissect on a consecutive section stained with haematoxylin alone and without a coverslip (right). Microdissection of areas of normal lymphocytes (N), dysplasia (1), adenocarcinoma (2) and mucinous cancer (3) was performed using a 27-G needle under an optical microscope at × 40–100 magnification. The two insets at the lower right (100×) show the area from which the largest dysplastic gland and the colloid mass from which a few nests of cancer cells were microdissected. The lower left panel shows the analysis of these microdissected areas for chromosome 17p and 18q allelic losses. Cancer cells of the colloid areas that form the bulk of the tumour show the loss of both chromosome 17p and 18q (lanes 3). The allelic loss of chromosome 17p was already present in a small proportion of cells among dysplastic (lane 1) and glandular carcinoma (lane 2) areas, as demonstrated by the diminished intensity of the lower allele with respect to the normal one (lane N) in the presence of upper alleles of comparable intensity in normal and neoplastic cells. The loss of the lower allele of chromosome 18q is already visible in the glandular carcinoma cells. These data suggest that the tumour bulk has been formed by a subclone with growth advantage that was already present at the stage of dysplasia

panel, it is worth noting that all but one of the 13 patients whose cancer showed concurrent chromosome 17p and 18q LOH died from disease.

A significant suggestion from this study is that for routine diagnostic purposes the RER phenotype can be ascribed using as few as 50–200 microdissected cells and only one or two mononucleotide markers, such as BAT-26 and BAT-40. The use of paraffin-embedded tissues allows selection of the most representative neoplastic areas as exemplified in *Fig. 4*, as well as of normal cells in the tumours for which only cancer tissue is available. In fact, lymphocytes, adipocytes or a blood vessel wall are adequate as a source of matched normal DNA, eliminating confusion between a rare polymorphism and a false-positive case[25]. The demonstration that only BAT-26 and/or BAT-40 is sufficient to detect the RER+ phenotype is in agreement with the findings of other recent reports[7,19,25].

In conclusion, although based on a relatively limited number of cases, the present study suggests that RER status has prognostic significance among patients with advanced gastric cancer undergoing curative surgery. Moreover, this information might have therapeutic implications, as it has been demonstrated recently that MSI cell lines are resistant to platinum drugs used in adjuvant treatment of gastric cancer[34] but show decreased survival after exposure to ionizing radiation[35].

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Original article

Lymph node micrometastasis and prognosis in patients with oesophageal squamous cell carcinoma

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Background: The purpose of this study was to investigate whether the presence of lymph node micrometastasis in pathological lymph node-negative (pN_0) oesophageal squamous cell carcinoma had prognostic value.

Methods: Some 1840 lymph nodes were obtained from 50 patients with pN_0 oesophageal squamous cell carcinoma who underwent curative resection of the primary tumour with systematic lymphadenectomy. These lymph nodes were examined immunohistochemically with anticytokeratin antibody (AE1/AE3). Lymph node micrometastases newly detected by immunohistochemistry were classified as micrometastasis. Additionally, lymph node micrometastases were classified into three stages: stage 1, one individual AE1/AE3-positive cell; stage 2, multiple individual positive cells; stage 3, one or multiple positive clusters.

Results: Micrometastases were detected in 20 patients (40 per cent). A higher stage of micrometastasis was associated with greater pathological tumour (pT) size ($P = 0.023$). Recurrent tumours developed in nine patients. However, the frequency of recurrence was similar in patients with, or without, micrometastasis (five of 20 and four of 30 patients respectively; $P = 0.25$). Twenty-three of 30 patients without micrometastasis survived, whereas 15 of 20 patients with micrometastasis were still alive (5-year overall survival 75 and 78 percent respectively, $P = 0.91$). Twenty-six of 30 patients without micrometastasis had no recurrence, whereas 15 of 20 patients with micrometastasis had no recurrence (5-year relapse-free survival 86 and 73 per cent respectively, $P = 0.37$). There was no significant difference in prognosis with respect to the stages of micrometastasis. Multivariate analysis also showed that micrometastasis was not an independent prognostic factor ($P = 0.73$).

Conclusion: Immunohistochemical detection of lymph node micrometastasis may be an indicator of lymphatic dissemination of tumour cells. However, the presence of micrometastasis had no impact on the prognosis of node-negative patients with oesophageal squamous cell carcinoma.

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Introduction

Although there have been recent improvements in surgical procedures, the overall prognosis for patients with oesophageal carcinoma remains poor, with 5-year survival rates of 5–45 per cent[1–3]. Some reports using multivariate analysis have revealed lymph node metastasis to be an independent prognostic factor[4,5].

Patients with oesophageal cancer without nodal metastasis have a lower rate of recurrence after operation than those with nodal metastasis[6,7]. However, relapse rates in patients with pathological node-negative (pN_0) disease (range 4·7–42 per cent)[6–11] remain significant. Of four papers concerned with lymph node micrometastasis in oesophageal cancer, three[12–14] emphasized its prognostic value. However, one report[8] found opposing results. The number of patients and dissected lymph nodes may have been too few to gain a definitive answer.

The purpose of this study was to investigate whether lymph node micrometastasis in patients with pN_0 tumours had prognostic value. For this purpose, a large number of nodes was examined immunohistochemically. The clinical impact of lymph node micrometastasis on prognosis was determined.

Patients and methods

Patient selection

Between January 1988 and June 1997, 258 patients with primary oesophageal cancer were treated at the Department of Surgery and Surgical Basic Science of Kyoto University. Some 211 consecutive patients underwent resection of the primary cancer with systematic lymphadenectomy by the same surgeon[15]. Sixty-five patients had no lymph node metastasis (pN_0) detected by routine pathological examination with haematoxylin and eosin staining. However, 15 of these patients were excluded from the study for the following reasons: three patients did not undergo curative resection, eight had synchronous cancer in another organ, three had tumours other than squamous cell carcinoma, and there was one operative death. No other patient died in hospital.

Lymphadenectomy

Details of the surgical procedure have been described previously[15]. Two types of systematic lymphadenectomy were performed on patients in this study: two-field lymphadenectomy in 30 patients and three-field lymphadenectomy in 20 patients. The criteria for a three-field lymphadenectomy were: no major preoperative or intraoperative complications; curative resection expected as a result of preoperative and intraoperative diagnosis; cervical lymph node metastasis suspected by preoperative imaging; cervical and upper thoracic oesophageal cancer; intraoperative frozen section of the paratracheal lymph node was positive in a patient with middle or lower thoracic oesophageal cancer. Patients who did not satisfy these criteria had a two-field lymphadenectomy.

Some 1840 lymph nodes were obtained from 50 patients (mean 37 (range 6–136) nodes per patient). Historical formalin-fixed and paraffin-embedded specimens of primary tumours and lymph nodes were studied immunohistochemically. Primary cancers of the oesophagus were classified according to the pathological tumour node metastasis (pTNM) classification[16].

Antibodies

Initially, three antibodies were tested as epidermal cell markers: anti-cytokeratin antibody cocktail (AE1/AE3), Ber-EP4 and anti-epithelial membrane antigen (EMA) antibody (Dako, Glostrup, Denmark). In the series, immunoreactivity of Ber-EP4 and anti-EMA antibody to the normal oesophageal epithelium and the primary cancer were weak and heterogeneous. Thus, AE1/AE3 was selected for use; the reliability of the AE1/AE3 antibody has been described elsewhere[17].

Immunohistochemistry

The archival tissue samples were fixed in 10 per cent buffered formalin and embedded in paraffin. The paraffin sections were dewaxed, hydrated and treated using an autoclave heating antigen retrieval technique (soaking in citric acid 10 mmol/l solution (pH 6·0) at 120°C for 5 min). The endogenous peroxide activity was blocked with 0·3 per cent hydrogen peroxide in absolute methanol for 30 min. Non-specific staining was eliminated by 30 min incubation with 10 per cent normal horse serum. Excess normal serum was removed, replaced by the primary antibody, and incubated overnight at 4°C. After rinsing the slides, the sections were incubated with a 1:200 dilution of the secondary antibody, followed by avidin–biotin–peroxidase complex (Vecstain® Elite ABC Kit; Vector Laboratories, Burlingame, California, USA) for 40 and 50 min respectively. Subsequently, the sections were stained with 0·003 per cent 3,3-diaminobenzidinetetrahydrochloride and 0·005 per cent hydrogen peroxide in 0·05 mol/l Tris–hydrochloric acid buffer (pH 7·2), counterstained with Mayer's haematoxylin, dehydrated and mounted. Normal oesophageal epithelium was used as a positive control.

Definition and staging of lymph node micrometastasis

In the routine pathological examination, pathologists in this institution examined dissected lymph nodes by one maximum cross-section using haematoxylin and eosin staining. In this study, one extra cross-section, as close as possible to the section observed in the routine study, was stained. All newly detected lymph node metastases were defined as micrometastases. Lymph node micrometastases were classi-fied into four stages as follows: stage 0, no AE1/AE3-positive cells detected; stage 1, an individual cancer cell detected; stage 2, multiple individual cancer cells detected; stage 3, a cancer cluster or multiple cancer clusters detected (none found by haematoxylin and eosin staining). Two authors judged micrometastasis in a blinded fashion. AE1/AE3-positive cells in four lymph nodes from two patients were confirmed as macrophages by CD68 staining, and excluded.

Follow-up

None of the 50 patients received adjuvant postoperative treatment. After relapse of the tumour, patients received various treatments, including chemotherapy, radiation therapy and surgical resection. Follow-up information was obtained by direct questioning of the patients, their families and physicians. Overall survival was determined from the time of operation to the time of death or last follow-up. Relapse-free survival was determined from the time of operation to the time of diagnosis of recurrence or last follow-up. The mean follow-up period was 67 (range 7–136) months.

Statistical analysis

Statistical calculations were performed using JMP® software version 3 for Macintosh (SAS Institute, Cary, North Carolina, USA). Proportional analysis between the sample groups was performed with Fisher's exact probability test in 2×2 contingency tables, and with the χ^2 test or Kruskal–Wallis test in $n \times n$ contingency tables. The Kaplan–Meier survival model was used to estimate overall and relapse-free survival. The log rank test and generalized Wilcoxon test were used to determine statistical differences between groups. Cox's proportional hazard model was fitted to multivariate analysis. All tests were two sided, and the results were considered significant when $P < 0{\cdot}05$.

Results

Immunoreactivity for AE1/AE3 in lymph node metastasis

The anticytokeratin antibody AE1/AE3 recognizes many types of cytokeratin[17]. Its staining was observed in the cytoplasm of the cancer cell and normal oesophageal epithelial cells. All primary tumours were stained intensely by AE1/AE3 (*Fig. 1*).

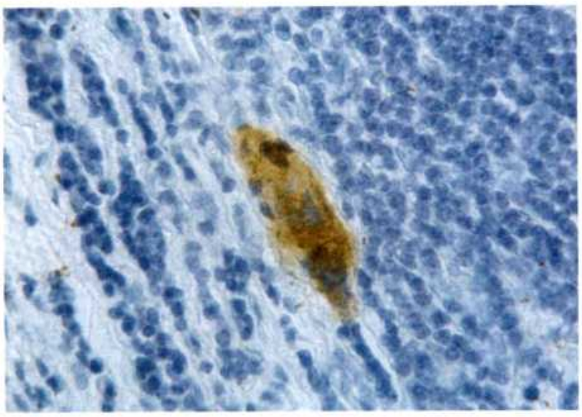


Fig. 1 Immunostaining of lymph node micrometastasis by AE1/AE3 antibody. A cancer cell cluster in the peripheral sinus was stained; this micrometastasis was classified as stage 3

Detection of micrometastases and the correlation between micrometastases and clinicopathological findings

Micrometastases were detected in 20 (40 per cent) of the 50 patients, and in 29 (2 per cent) of 1840 lymph nodes examined. Clinicopathological details of the patients are shown in *Table 1*; no statistical association was found for the presence of micrometastasis.

A higher stage of micrometastasis was associated with greater tumour size (*Table 2*). Moreover, patients with a higher pT rating tended to have more lymph node micrometastases ($P = 0{\cdot}069$).

Impact of lymph node micrometastasis on prognosis

Twenty-three of 30 patients without micrometastasis (mean follow-up 54 months) survived, whereas 15 of 20 patients with micrometastasis (mean follow-up 63 months) were still alive (5-year overall survival 75 and 78 per cent respectively, $P = 0{\cdot}91$) (*Fig. 2a*). Twenty-six of 30 patients without micrometastasis had no recurrence, whereas no recurrence was developed in 15 of 20 patients with micrometastasis (5-year relapse-free survival 86 and 73 per cent respectively, $P = 0{\cdot}37$) (*Fig. 2b*). The presence of lymph node micrometastasis did not appear to be associated with overall survival or relapse-free survival.

Table 1 Clinicopathological details of 50 patients who had resection of oesophageal squamous cell carcinoma

	Lymph node micrometastasis		
	Absent ($n=30$)	Present ($n=20$)	P
Sex ratio (M : F)	21 : 9	15 : 5	0·76*
Mean(s.d.) age (years)	66·1(9·2)	61·9(8·6)	0·11†
Location of primary tumour			
Cervical	1	0	0·17‡
Upper thoracic	5	0	
Middle thoracic	20	15	
Lower thoracic	4	5	
Tumour size (pT stage)			
pT_1	17	9	0·24‡
pT_2	6	2	
pT_{3-4}	7	9	
Histology (grade)			
Well differentiated	7	6	0·83‡
Moderately differentiated	16	9	
Poorly differentiated	7	5	
Lymphadenectomy			
Two-field	20	10	0·26*
Three-field	10	10	
Mean(s.d.) no. of dissected lymph nodes	34(17)	40(10)	0·17†

pT, pathological tumour. *Fisher's exact test; †Student t-test; ‡χ^2 test

Table 2 Micrometastasis and tumour size

	pT_1	pT_2	pT_{3-4}	P*
Stage of micrometastasis				
1	7	0	2	
2	1	0	3	0·023
3	1	2	4	
No. of micrometastases				
0	19	7	7	
1	8	2	4	
2	0	0	4	0·069
3	1	0	0	
4	0	0	1	

pT, pathological tumour. *Kruskal–Wallis test

The clinical impact on prognosis of using other criteria of lymph node micrometastasis, either by staging (stage 0–1 *versus* stage 2–3) or by number of micrometastases (0–1 micrometastasis *versus* 2–4 micrometastases), did not reveal any statistical difference in patients' prognosis.

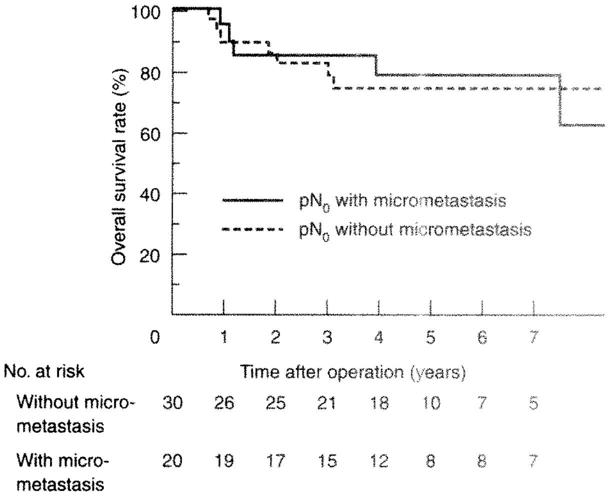
According to Cox regression analysis, tumour size (pT factor) of the pTNM classification was most independently associated with overall survival, followed by sex. As none of the women in this series developed recurrent carcinoma, sex was the strongest independent factor for relapse-free survival, followed by pT factor. Micrometastasis did not have any independent effect on overall or relapse-free survival (*Table 3*).

Pattern of recurrence

Twelve recurrent tumours were found in nine patients. These tumours included five cervical lymph node recurrences, five mediastinal lymph node recurrences, one local recurrence and one haematogenous recurrence. The incidence of recurrence in patients with and without micrometastasis was five of 20 and four of 30 respectively ($P = 0·25$). Two patients with cervical lymph node recurrence remain alive following additional resection of recurrent tumour. The other seven patients died from recurrent tumour.

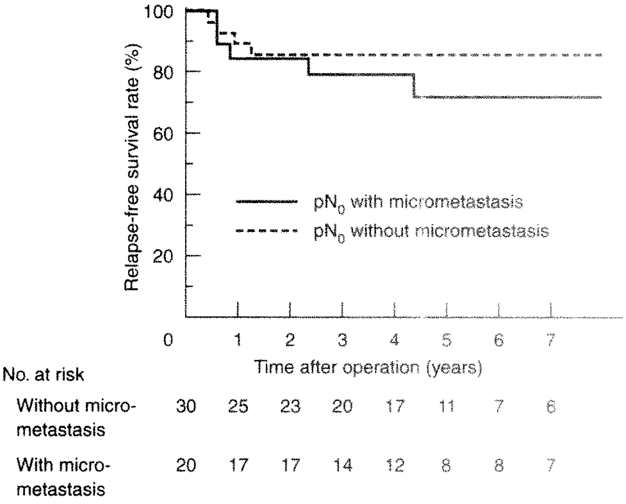
Discussion

The prognosis in patients with oesophageal cancer remains poor and is known to be correlated with the presence of lymph node metastasis. However, the relapse rate even in node-negative patients (range 4·7–42 per cent)[6–11] remains a problem. As cancer cell detection methods have developed[18], micrometastases have been detected in patients that were not found by conventional histological examination. Four previous authors[8,12–14] investigated whether lymph node micrometastasis of oesophageal cancer had clinical significance. Three[12–14] of the four authors emphasized the prognostic value of micrometastasis. By contrast, this study showed no significant survival difference, despite the presence of micrometastasis.



No. at risk	0	1	2	3	4	5	6	7
Without micrometastasis	30	26	25	21	18	10	7	5
With micrometastasis	20	19	17	15	12	8	8	7

a Overall survival



No. at risk	0	1	2	3	4	5	6	7
Without micrometastasis	30	25	23	20	17	11	7	6
With micrometastasis	20	17	17	14	12	8	8	7

b Relapse-free survival

Fig. 2 Kaplan–Meier curves showing **a** overall survival rate and **b** relapse-free survival rate according to the presence or absence of lymph node micrometastasis in 50 patients who had surgical resection of a lymph node-negative squamous cell carcinoma of the oesophagus. **a** $P = 0·91$ (log rank test), $P = 0·73$ (Wilcoxon test); **b** $P = 0·37$ (log rank test), $P = 0·46$ (Wilcoxon test)

Possible reasons for this discrepancy can be identified by evaluation of previous studies (*Table 4*). The first point is the different clinical backgrounds of patients with negative lymph nodes among these studies. Although tumour size was similar in the previous studies, the mean number of

Table 3 Multivariate analysis for prognosis after resection of node-negative oesophageal squamous cell carcinoma

	Overall survival		Relapse-free survival	
	Risk ratio	P*	Risk ratio	P*
Sex (M, F)	0·11 (0·00–0·87)	0·035	0·00 (0·00–0·00)	0·003
Age (< 64 years, ≥ 64 years)	1·25 (0·29–6·28)	0·77	1·16 (0·23–5·49)	0·85
Location (cervical, upper thoracic, middle, lower thoracic)	0·13 (0·01–1·33)	0·082	0·10 (0·01–0·94)	0·045
Tumour size (pTNM) (pT_1, pT_{2-4})	23·6 (3·48–515·3)	0·0004	7·02 (1·12–67·6)	0·037
Grade (differentiation) (well, moderate, poor)	0·88 (0·09–6·67)	0·91	2·90 (0·40–19·5)	0·28
Lymphadenectomy (two-field, three-field)	0·96 (0·23–3·84)	0·95	4·14 (0·96–22·7)	0·057
Micrometastasis (absent, present)	0·77 (0·15–3·48)	0·73	0·93 (0·12–8·14)	0·94

Values in parentheses are 95 per cent confidence intervals. pTMN, pathological tumour node metastasis. *Cox's proportional hazard test

Table 4 Previous reports on micrometastasis in patients with node-negative oesophageal cancer

					2-year overall survival rate (%)	
Reference	Year	T_{2-4} per total pN_0*	No. of lymph nodes†	Micrometastasis positive*	pN_0 without micrometastasis	pN_0 with micrometastasis
Izbicki *et al.*[12]	1997	—	680 (10)‡	15 of 30 (50)	—	—
Natsugoe *et al.*[13]	1998	17 of 41 (41)	1954 (24)‡	15 of 41 (37)	85¶	38¶
Glickman *et al.*[8]	1999					
Adenocarcinoma		25 of 49 (51)	341 (7)§	15 of 49 (31)	55	58
Squamous cell carcinoma		13 of 29 (45)	233 (8)§	5 of 29 (17)	88	89
Komukai *et al.*[14]	2000	22 of 37 (59)	2845 (75)§	14 of 37 (38)	91¶	50¶
Present study	2001	24 of 50 (48)	1840 (37)†	20 of 50 (40)	86	85

Values in parentheses are *percentages and †mean no. per patient. pN, pathological node; number of dissected lymph nodes is given for patients with ‡pN_0 or pN_1 disease, or §pN_0 disease. ¶Survival rates were estimated from values in the literature because 2-year survival rates were not described definitely

lymph nodes examined varied considerably (10, 24, 7, 8 and 75 nodes per patient). In the present study, a mean of 37 nodes was examined per patient. Wong *et al.*[19] reported that the number of examined nodes influenced staging accuracy in colorectal carcinoma. Izbicki *et al.*[12] examined only 680 nodes using immunohistochemistry of a total of 1308 dissected lymph nodes evaluated by haematoxylin and eosin staining. This may have led to selection of a biased pN_0 group. Additionally, Izbicki *et al.*[12] did not describe the impact of lymph node micrometastasis in patients with pN_0 tumours. Glickman *et al.*[8] detected lymph node micrometastasis in only 31 per cent (adenocarcinoma) and 17 per cent (squamous cell carcinoma) of node-negative patients, probably because of the low number of nodes examined. Detection rates of micrometastasis were similar (37–40 per cent) in the present study and in that of Natsugoe *et al.*[13] and Komukai *et al.*[14]. This suggests that there was no difference in the sensitivity of the detection methods and clinical features in these three studies.

Second, the definition of lymph node micrometastasis varied between the reports. In the present report, lymph node micrometastases were classified into three stages. Natsugoe *et al.*[13] emphasized the importance of a stromal reaction. All stage 3 micrometastases in the present series had a stromal reaction. However, there was no difference in prognosis between stages 1–2 and stage 3. Three authors set definite criteria for micrometastasis size; those described by Glickman *et al.*[8], Natsugoe *et al.*[13] and Komukai *et al.*[14] were 'less than 2 mm in diameter', 'less than 0·5 mm in diameter' and 'equal to, or less than five cancer cells' respectively. All micrometastases detected in the present study were less than 0·5 mm in diameter, but included clusters with more than five cells. The series of Glickman *et al.* might have included patients with pN_0 disease whose micrometastases could have been detected by careful haematoxylin and eosin examination. By contrast, Komukai *et al.*'s series might have excluded patients with metastasis that was difficult to detect by ordinary haematoxylin and eosin staining. Despite careful assessment of the effect of defining micrometastases by various criteria, it was difficult to find any significant impact.

Several authors[3,9,20–23] have described how the number of lymph node metastases determined by haematoxylin and eosin staining, or the ratio of involved to removed lymph nodes, was associated with the prognosis or rate of locoregional recurrence. The number of micrometastases did not appear to influence overall survival or relapse-free survival in the present study.

According to univariate and multivariate analysis, lymph node micrometastasis had no significant impact on prognosis in the present study. Cox regression analysis revealed that tumour size was the strongest independent factor affecting prognosis ($P = 0{\cdot}0004$). This result was consistent with previous studies employing conventional histological examination[1]. The second prognostic factor was sex ($P = 0{\cdot}035$). According to survival data[4] from the entire group of patients with oesophageal cancer treated at this institution, both sex and node status correlated with prognosis. As node-positive patients were excluded in this study, it was not surprising that female sex remained associated with good prognosis.

Izbicki *et al.*[12] discussed how lymphatic dissemination of tumour cells could be an earlier event than bloodborne spread. Lymph node micrometastases were detected even in pT_1 stage tumours in this, and previous, studies. Furthermore, pT factor was associated with the number and stage of lymph node micrometastases in the present study, suggesting that lymphatic dissemination of tumour cells might start in early T stage. The number and stage of micrometastases increased as the tumour enlarged.

Another reason that the presence of micrometastases did not appear to affect outcome in the present study was that survival of patients with pN_0 tumours with micrometastasis was relatively better than that in the other studies. The policy of systematic lymphadenectomy may have had an impact on prognosis, even when there were micrometastases. If lymph nodes were not dissected, there may have been a negative impact on survival when micrometastasis was present, but the effect could not be seen because micrometastases were removed effectively in the early phase of lymphatic dissemination.

In this study the detection of lymph node micrometastases had no statistical effect on outcome. However, this and all previous studies were retrospective. Therefore, a larger prospective study is needed to resolve this question.

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Original article

Gastrointestinal complications in lung transplant survivors that require surgical intervention

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Background: Lung transplantation is widely accepted as a treatment for end-stage lung disease. At present, information regarding the incidence and outcome of acute gastrointestinal complications in lung transplant survivors is limited.

Methods: Since 1990, 127 lung transplantations have been performed in 125 patients: 73 males (58 per cent) and 52 females (42 per cent) of median age 43 (range 9–64) years. Patients received a standard induction and maintenance regimen of immunosuppression.

Results: At a median follow-up of 2·6 (range 0–8·6) years the overall survival rate was 68 per cent. An acute abdomen requiring surgical intervention was diagnosed in 12 patients (10 per cent). The median time following lung transplantation was 19 (range 3–68) months. Eight cases of bowel perforation, two of appendicitis, one of colitis, one of cholecystitis, and one pneumoperitoneum were encountered. Four Hartmann procedures, two sigmoid resections, one small bowel resection, two appendicectomies, a subtotal colectomy, a cholecystectomy and an exploratory laparotomy were performed with minimal morbidity and no postoperative death.

Conclusion: Lung transplant survivors are at increased risk of developing an acute abdomen because of the use of high-dose immunosuppressive agents. Physicians who evaluate lung transplant patients for an acute abdomen should have a low threshold for surgical intervention.

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Introduction

Over the past decade the outcome of lung transplantation has improved. According to the report[1] of the Registry of the International Society for Heart and Lung Transplantation, published in 1998, there have been 7021 lung transplantations performed with a 1-year survival rate approaching 75 per cent and a 5-year survival rate slightly greater than 40 per cent. Lung transplantation will continue to be a viable treatment for patients with end-stage lung disease.

As more lung transplant patients survive the acute perioperative period, more long-term complications are being encountered. Surgical and non-surgical gastrointestinal complications have been described after solid-organ transplantion, with various clinical outcomes[2–9]. There are limited data concerning gastrointestinal complications in survivors of lung transplantation[10–15]. This report describes the incidence of acute abdomen requiring surgical intervention in lung transplant survivors treated at Groningen University Hospital.

Patients and methods

A total of 127 lung transplantations, including two retransplantations, have been performed in 125 patients, 73 males (58 per cent) and 52 females (42 per cent) of median age 43 (range 9–64) years, at Groningen University Hospital between November 1990 and March 1999. The clinical syndrome of an acute abdomen was diagnosed on 13 occasions in 12 patients.

After lung transplantation patients received intense quadruple immunosuppressive therapy consisting of cyclosporin, azathioprine, prednisolone and rabbit antithymocyte globulin as induction therapy. The post-transplant maintenance immunosuppressive regimen consisted of azathioprine (1–3 mg per kg per day), cyclosporin A (dosage titrated to maintain initial whole blood trough levels of 400 µg/l, tapering to 150 µg/l in 3 weeks) and prednisolone (0·2 mg per kg per day for 6 months, 0·1 mg per kg per day thereafter). Patients were followed closely in the Groningen University Hospital Transplant Centre. Close communication was maintained between primary care and lung transplant physicians. Evaluation, investigation and surgical intervention were all performed at Groningen University Hospital, except for one patient who was treated elsewhere.

Results

The median follow-up of the 125 patients was 2·6 (range 0–8·6) years with an overall survival rate of 68 per cent. There were 13 episodes in which a diagnosis of acute abdomen was made, in 12 lung transplant patients (10 per cent of the total lung transplant population). The median age of these patients was 46 (range 12–63) years, and the median time from lung transplant surgery to the acute abdominal intervention was 19 (range 3–68) months. *Table 1* summarizes the underlying causes of the acute abdomen.

In eight cases the acute abdomen was caused by bowel perforation: five sigmoid perforations resulting from diverticulitis, one perforation of a Meckel diverticulum, one sigmoid perforation with an enterocutaneous fistula, and one ileal perforation. In one of these patients the bowel perforation occurred at the time of an acute rejection episode. Four Hartmann procedures, two sigmoid resections with a primary anastomosis, one small bowel resection and one hemicolectomy with ileostomy were performed.

In addition there were two cases of acute appendicitis, one case of acute colitis without an underlying diagnosis and one case of cholecystitis diagnosed and treated with appendicectomy, a colectomy with an ileostomy, and a laparoscopic cholecystectomy. One patient with epigastric pain and free air under the diaphragm underwent explorative laparotomy in which no gastrointestinal perforation was found. However, the patient subsequently died from an adult respiratory distress syndrome-type illness. There was no autopsy performed.

Table 1 Primary underlying diagnoses resulting in an acute abdomen in 125 lung transplant patients

Diagnosis	No. of patients (*n* = 125)
Bowel perforation	8 (6)
Appendicitis	2 (2)
Cholecystitis	1 (1)
Colitis	1 (1)
Pneumatosis intestinalis	1 (1)
Total	13 (10)

Value in parentheses are percentages

Concomitant post-transplant lymphoproliferative disorder (PTLD) was diagnosed in three patients. There were two cases of PTLD that presented as a bowel perforation. In one patient with appendicitis, multiple metastases of PTLD to the liver were diagnosed. All these patients required further treatment for the underlying PTLD process.

Patient characteristics, the individual diagnoses and the interventions performed are presented in *Table 2*. There was no significant difference in age or sex between the patients with or without an acute abdomen. In this series of 13 urgent laparotomies there was no postoperative death. The postoperative morbidity consisted of a colovesicle fistula that required surgical correction, a laparotomy wound dehiscence that required operation and a subglottic stenosis treated by means of tracheostomy.

Discussion

The lung transplant patient population in this series had a high incidence (10 per cent) of the clinical syndrome of acute abdomen. There have been relatively few reports of the incidence of gastrointestinal complications and associated death in patients undergoing lung, heart–lung and heart transplantation. These reports are summarized in *Table 3*[2,4,5,8,10–15]. The incidence of gastrointestinal complications varied in these series between 4 and 17 per cent, with a mortality rate of 0–63 per cent. Inflammatory disease and PTLD seem to have a substantial role in the generation of gastrointestinal complications in lung transplant survivors.

Bowel perforation as a consequence of diverticulitis or PTLD is encountered frequently. The cause of the increased incidence appears to be multifactorial. The most likely contributing factor is the chronic steroid component of the immunosuppressive regimen[2,4,5,7,10–14,16–20]. Although the pathophysiological mechanism has not been clearly delineated, postulated mechanisms for the correlation of steroid use and bowel perforation include inhibition of the normal processes of mucosal repair by steroids, a direct steroidal injury to the mucosa, thinning of the bowel wall caused by atrophy of the submucosal lymphoid patches, and inhibition of the bowel's ability to ward off invasion by bacteria[10,17,18,20]. Larger doses of steroids over longer periods of time may correlate with a longer time to surgical intervention and an increased patient mortality, as these patients present abnormally without the guarding or rebound tenderness expected in acute abdomen[17,18]. In patients on chronic steroid therapy, the presence of pneumoperitoneum may be the sole indication of an acute abdominal process[17,18,20,21].

Table 2 Summary of lung transplant recipients diagnosed with an acute abdomen that required surgical intervention

Patient no.	Age at time of abdominal complication (years)	Sex	Primary diagnosis	Transplant performed	Time of abdominal surgery post-transplant (months)	Diagnosis and type of operation
1	57	F	α_1-AT deficiency	Bilateral	55	Diverticulitis with sigmoid perforation; sigmoid resection, primary anastomosis
2	44	M	α_1-AT deficiency	Bilateral	29	Ileal perforation; ileal resection
3*	12	M	Cystic fibrosis	Bilateral	10	Colitis; subtotal colectomy with ileostomy
4	46	M	α_1-AT deficiency	Bilateral	19	PTLD, diverticulitis with sigmoid perforation; sigmoid resection, Hartmann procedure
5	47	M	PPH	Unilateral	38	Diverticulitis with sigmoid perforation; sigmoid resection, Hartmann procedure
6*	61	M	Fibrosing alveolitis	Bilateral	4	Exploratory laparotomy
7	48	M	α_1-AT deficiency	Bilateral	68	Appendicitis and metastatic PTLD in liver; appendicectomy
8	43	F	α_1-AT deficiency	Bilateral	5	Diverticulitis with sigmoid perforation; sigmoid resection, Hartmann procedure
9	26	F	Cystic fibrosis	Bilateral	8	Cholecystitis; laparoscopic cholecystectomy
	28				48	Appendicitis and endometrial cyst rupture; appendicectomy and laparoscopic left oophorectomy
10	49	F	Bronchiectasis	Bilateral	32	PTLD and perforation of Meckel diverticulum; ileal resection
11*	63	M	Emphysema	Bilateral	16	Diverticulitis with sigmoid perforation and fistula; sigmoid resection with double loop colostomy
12	44	M	α_1-AT deficiency	Bilateral retransplant	3	Diverticulitis with sigmoid perforation; sigmoid resection, Hartmann procedure

*Patient has since died from cause other than acute abdominal complication. α_1-AT, α_1-antitrypsin; PPH, primary pulmonary hypertension; PTLD, post-transplant lymphoproliferative disorder

In the Groningen University lung transplant population, the overall prevalence of PTLD in the present series, with a median follow-up of 2·6 years, is 9 per cent. The high incidence of PTLD in lung transplant patients (4·5–10 *versus* 1·7 per cent in transplant patients as a whole) may be related to the more intense immunosuppression[22]. In addition, the standard immunosuppressive therapy used in transplant patients may have more than one mechanism for promoting malignancy. It has recently been shown that cyclosporin, in addition to promoting tumour proliferation by suppressing the body's own protective immune response, may act directly on tumour cells through a transforming growth factor β-dependent system to induce progression and invasion[23]. However, it is currently unknown whether cyclosporin has an effect in converting cells from a benign to a malignant state. Although the incidence of PTLD in transplant patients has been documented, the incidence of PTLD affecting the small and large bowel is unclear. One study of 1385 heart, lung and kidney recipients found an overall PTLD incidence of 1·7 per cent. Of these cases, 25·0 per cent involved the gastrointestinal tract and only 8·3 per cent resulted in major abdominal surgery[22]. Another study of 219 lung transplant patients reported four cases of PTLD (1·8 per cent); only one involved the gastrointestinal tract and led to perforation[24].

The overall incidence of colonic perforation may be higher in lung transplant recipients than in recipients of other solid organs[10–12,14]. One series reported an incidence of 6·7 per cent in lung transplant recipients, compared with a calculated 1–2 per cent incidence in renal, liver and heart–lung recipients[10]. The incidence of bowel perforations in different lung transplant series varied between 5 and 20 per cent[10–12,14]. The morbidity and mortality rates in these cases may be reduced by rapid recognition of abdominal

Table 3 Review of the incidence and mortality of cases of acute abdomen in lung, heart–lung and heart transplant patients

				Acute abdomen		Acute abdomen > 1 month post-transplant	
Reference	Year	Total no. of patients	Type of transplant	Incidence (%)	Mortality (%)	Incidence (%)	Mortality (%)
Steed *et al.*[8]	1985	143	Heart, heart–lung, heart–liver	6	44	5	29
Merrell *et al.*[5]	1989	178	Heart	10	0	—	—
Kirklin *et al.*[4]	1990	151	Heart	8	25	—	—
Augustine *et al.*[2]	1991	117	Heart, heart–lung	9	9	8	10
Smith *et al.*[14]	1995	75	Lung	12	—	8	—
Beaver *et al.*[10]	1996	60	Lung	7	50	5	33
Lubetkin *et al.*[12]	1996	47	Lung	17	63	6	33
Fenton and Cicale[11]	1997	35	Lung	9	0	—	—
Pollard *et al.*[13]	1997	112	Lung	4	50	—	—
Wekerle *et al.*[15]	1997	124	Lung	8	50	5	33
Present study	2001	125	Lung	10	0	10	0

pathology during initial evaluation and expeditious intervention, including diagnostic exploratory laparotomy. It is generally believed that, in case of a sigmoid perforation, sigmoid resection with a Hartman procedure is superior to resection with primary anastomosis. It offers minimal surgical manipulation and a shorter intraoperative time interval, resulting in low morbidity and mortality[5,9,10,17,18].

Appendicitis is the most frequent cause of an acute abdomen in the normal population. The incidence of appendicitis in post-transplant patients has not been reviewed frequently. Review of appendicitis in renal transplants showed that, although the immunosuppressive regimen and older age of the patients may mask the classical presentation and delay the correct diagnosis, the morbidity and mortality due to appendicitis is much lower than that seen with other gastrointestinal complications, such as diverticulitis[6,25].

Transplant patients have an increased risk of developing cholelithiaisis and cholecystitis. Cholelithiasis has been associated with the immunosuppressive drug cyclosporin and a cholestatic effect of cyclosporin on the liver[26]. It has also been postulated that obesity and weight fluctuations with steroid therapy and the older age of transplant patients are further contributing factors[27]. In lung transplantation, the diagnosis of cystic fibrosis is another contributory risk factor, as the disease itself is associated with cholelithiasis. The diagnosis of acute cholecystitis in a lung transplant patient may be difficult to discern. The incidence of symptomatic gallbladder disease that requires surgical intervention ranges in the literature from 1·3 to 3·8 per cent in heart and heart–lung transplant patients, and from 1·4 to 4·5 per cent in lung transplant patients[2–5,8,12–15,28]. There have been two studies[29,30] reporting the outcome of surgical biliary complications in heart transplant patients. The high mortality rates of 14 and 36 per cent were related partly to the increased likelihood of septic complications in steroid-dependent immunosuppressed patients and partly to the atypical presentations that masked the underlying severity of the disease processes. Elective (laparoscopic) cholecystectomy may avoid the potential risks of acute cholecystitis[13,15,27]. It is unclear at this time whether cholecystectomy in asymptomatic cholelithiasis in lung transplant recipients is warranted[15].

The lung transplant patient with abdominal pain should be evaluated and treated like any other patient with the same symptomatology. Although most abdominal pain will resolve without surgical intervention, a thorough medical approach is warranted and diagnosis in these patients is a challenge. The decision-making algorithm about when and how to intervene in these patients with abdominal pain may sometimes be extremely difficult. A similar diagnostic dilemma is encountered in the neutropenic cancer patient with abdominal pain. Decreased immunocompetence may mask the signs and symptoms of a gastrointestinal catastrophe. Any doubt is a plea for operation, instead of observation.

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Original article

Other primary cancers occurring after treatment of superficial oesophageal cancer

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Background: Recent advances in endoscopic diagnosis and treatment have led to better prognosis for patients with superficial oesophageal cancer. The incidence of subsequent other primary cancer (SOPC) has become a new problem for patients who survive after treatment of superficial oesophageal cancer.

Methods: Between 1966 and 1998, 368 patients with superficial oesophageal cancer, histologically confirmed as squamous cell carcinoma after resection, were reviewed for the presence of SOPC.

Results: Among the 368 patients, 43 developed SOPC. The most frequent sites of SOPC were the stomach (11 patients) and hypopharynx (11). Subsequent cancers of the stomach and hypopharynx developed significantly more frequently in heavy smokers. The 5-year cumulative occurrence rate of subsequent cancers within the fields of endoscopy of the upper gastrointestinal tract (stomach, hypopharynx and residual oesophagus) was 15 per cent.

Conclusion: Gastric and hypopharyngeal cancers were frequently found after resection of superficial oesophageal cancer. A history of heavy smoking at the time of initial resection may be a risk factor. To make an early diagnosis of subsequent cancers, follow-up observation by upper gastrointestinal endoscopy is important after treatment of oesophageal cancer.

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Introduction

Squamous cell carcinoma of the oesophagus is often associated with malignancy of other organs such as that of the head and neck, upper respiratory tract and the rest of the alimentary tract[1–10]. Patients with oesophageal cancer seldom live long enough to develop a second cancer. Therefore, many cancers of other organs may have occurred before or synchronously. Few reports have focused on a sufficiently large series of patients to determine the incidence of subsequent other primary cancer (SOPC) occurring after treatment of oesophageal cancer.

Recent advances in endoscopic diagnosis have led to an increase in the number of patients with superficial oesophageal cancer who have a relatively good prognosis. Various advances and innovations in resection techniques and chemoradiation for superficial oesophageal cancer have increased the number of patients with prolonged postoperative survival[11,12]. In this group the incidence of SOPC has become a new problem. Another important issue is the increase in the incidence of multiple primary oesophageal cancer because of the more widespread use of endoscopic mucosal resection for superficial oesophageal cancer[13,14]. The present study was designed to determine the clinical features of SOPC occurring after treatment of superficial oesophageal cancer.

Patients and methods

Among the Japanese population, most cases of oesophageal carcinoma are of squamous cell origin. Between 1966 and 1998, 368 patients with superficial oesophageal cancer histologically confirmed as squamous cell carcinoma, who underwent surgical resection or endoscopic mucosal resection at National Cancer Centre Hospital, Tokyo, were reviewed. These patients had not undergone any preoperative therapy, and were followed for at least 6 months after resection. Upper gastrointestinal endoscopy was done every 6 months after endoscopic resection and every 12 months after surgical resection. The clinical characteristics of patients are summarized in *Table 1*.

Superficial oesophageal cancer has been defined as a lesion confined to the mucosa or submucosa, regardless of the presence or absence of metastases[15]. Multiple primary oesophageal carcinoma was identified histologically when each cancerous lesion was located individually, showing no continuity, and when definite primary malignant features were evident, excluding intramural metastasis of the main lesion[16].

A tumour was defined as synchronous if it occurred within 6 months of the diagnosis of the oesophageal cancer, antecedent if diagnosed more than 6 months before the oesophageal cancer, and subsequent if diagnosed more than 6 months afterwards[2]. The diagnosis of synchronous tumours was based on criteria described by Warren and Gates[17]. Differentiation between a non-oesophageal primary tumour and metastasis was aided by evaluation of the clinical course and histological characteristics, including different cell types, different degrees of differentiation, and identification of the tumour origin from its overlying epithelium.

The characteristics of patients with SOPC or subsequent multiple oesophageal cancer were compared with those of patients with a solitary tumour. To elucidate risk factors, a history of antecedent and/or synchronous other primary cancer and synchronous multiple oesophageal carcinoma as internal factors, and smoking and drinking habits as external factors, were evaluated. As an indicator of cumulative smoking, the Brinkman index (number of cigarettes per day × years of smoking) was calculated at the time of initial resection of the superficial oesophageal cancer. Patients with a high Brinkman index (1000 or higher) were defined as heavy smokers. With regard to alcohol drinking, habitual drinkers were defined as those who drank on more than 5 days per week.

Differences between groups were analysed statistically by the χ^2 test with Fisher's exact probability test, and by Student's *t* test. The percentage of patients with subsequent cancer was calculated according to the Kaplan–Meier method. The effect of selected variables on the development of SOPC was assessed using the log rank test and the Cox–Mantel test.

Table 1 Characteristics of 368 patients with superficial oesophageal cancer

Mean(s.d.) age (years)	61·9(8·9)
Sex ratio (M : F)	323 : 45
Resection	
Endoscopic resection	101 (27)
Surgery	267 (73)
Depth of invasion	
Mucosa*	176 (48)
Submucosa†	192 (52)
History of multiple other primary cancers‡	149 (40)
Antecedent	68 (18)
Synchronous	93 (25)
Synchronous multiple oesophageal cancers	90 (24)

Values in parentheses are percentages unless otherwise indicated. *Intraepithelial carcinomas and tumours invading the lamina propria mucosa or the lamina muscularis mucosa; †tumours invading the submucosa; ‡history of antecedent and/or synchronous multiple other primary cancers

Table 2 Other primary cancers associated with superficial oesophageal cancer

Site	Antecedent	Synchronous	Subsequent	Total
Head and neck				
Hypopharynx	7	23	11	41
Oropharynx	8	7	3	18
Larynx	8	3	0	11
Tongue	5	5	3	13
Oral cavity	8	3	3	14
Thyroid	2	6	0	8
Stomach	18	40	11	69
Lung	2	4	7	13
Colon and rectum	10	6	7	23
Liver	0	5	1	6
Skin	2	0	1	3
Breast	3	0	0	3
Urinary bladder	1	1	1	3
Prostate	1	1	1	3
Kidney	0	1	0	1
Pancreas	0	1	0	1
Testicle	1	0	0	1
Lymphoma	1	1	0	2
Total	77	107	49	233

Results

Among the 368 patients, SOPCs occurring after resection of superficial oesophageal cancer were found in 49 organs in 43 patients (12 per cent). The most frequent sites were the stomach (11 patients) and hypopharynx (11), followed by the colon (seven) and lung (seven) (*Table 2*). SOPCs were diagnosed a mean(s.d.) interval of 44·8(29·5) months after resection (stomach, 36·9(16·9) months; hypopharynx, 50·9(20·9) months; lung, 23·1(10·4) months; colon, 34·9(22·2) months). Overall, multiple other primary cancers associated with superficial oesophageal cancer were found in 169 patients (46 per cent).

Among patients with SOPC, there were significantly more heavy smokers with a high Brinkman index (1000 or higher) at the time of initial resection of superficial oesophageal cancer ($P < 0·01$). Furthermore, a history of antecedent and/or synchronous other primary cancer was significantly more common in patients with SOPC

($P < 0·05$). There was no difference in the incidence of synchronous multiple oesophageal carcinoma between patients with and those without SOPC (*Table 3*).

Among SOPCs of the stomach and hypopharynx (the most frequent sites of occurrence), all gastric cancers were detected by follow-up endoscopic examination and were T_1 tumours. Six of the 11 patients with gastric cancer underwent endoscopic mucosal resection and five underwent surgery. Four of the 11 hypopharyngeal cancers were detected by follow-up endoscopy and had a favourable clinical outcome.

Subsequent multiple primary oesophageal cancers occurring after initial resection were detected in 19 patients (5 per cent), a mean of 23·4(17·0) months later. There was no significant difference in clinical features between the patients with and without multiple tumours (*Table 4*). Thirteen of the latter patients underwent endoscopic mucosal resection, six underwent surgery and two had radiation therapy. Seventeen patients with subsequent multiple oesophageal cancer who were resected endoscopically or by surgery had superficial oesophageal cancers on histological examination.

Kaplan–Meier estimates of the proportions of patients with SOPC after initial resection as a function of time are shown in *Fig. 1*. Overall, the 5-year cumulative occurrence rate of SOPC was 15 per cent and that of subsequent multiple oesophageal cancer was 7 per cent. The 5-year cumulative occurrence rate of subsequent cancers within the fields of upper gastrointestinal endoscopy (stomach, hypopharynx and residual oesophagus) was 15 per cent.

No statistically significant difference was observed in overall survival after resection of superficial oesophageal

Table 3 Demographic characteristics of patients with and without subsequent other primary cancers

	SOPC (*n* = 43)	No SOPC (*n* = 325)	*P*‡
Mean(s.d.) age (years)	62·8(9·5)	61·7(8·8)	n.s.
Sex ratio (M : F)	41 : 2	282 : 43	n.s.
Heavy smoker*	24 (56)	108 (33)	0·006
Habitual drinker†	38 (88)	257 (79)	n.s.
History of multiple other primary cancers	24 (56)	125 (38)	0·044
Synchronous multiple oesophageal cancers	12 (28)	88 (27)	n.s.

Values in parentheses are percentages unless otherwise indicated. *Patients with a high Brinkman index (1000 or higher) at the time of initial resection of superficial oesophageal cancer; †patients who drink alcohol on more than 5 days per week; ‡χ^2 test. SOPC, subsequent other primary cancer; n.s., not significant

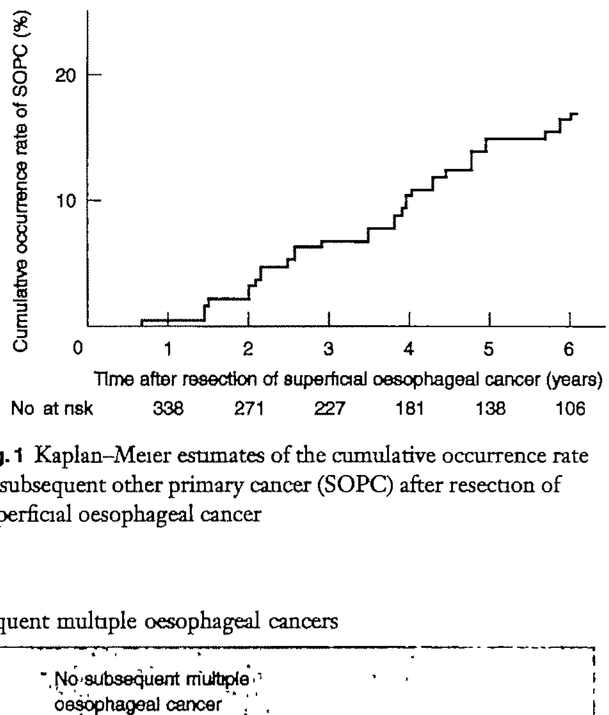

Fig. 1 Kaplan–Meier estimates of the cumulative occurrence rate of subsequent other primary cancer (SOPC) after resection of superficial oesophageal cancer

Table 4 Demographic characteristics of patients with and without subsequent multiple oesophageal cancers

	Subsequent multiple oesphageal cancer (*n* = 19)	No subsequent multiple oesophageal cancer (*n* = 349)	*P*‡
Mean(s.d.) age (years)	64·8(7·2)	61·7(9·0)	n.s.
Sex ratio (M : F)	17 : 2	306 : 43	n.s.
Heavy smoker*	6 (32)	126 (36)	n.s.
Habitual drinker†	13 (68)	282 (81)	n.s.
History of multiple other primary cancers	12 (63)	137 (39)	n.s.
Initial resection			0·0001
Endoscopic resection	13 (68)	88 (25)	
Surgery	6 (32)	261 (75)	

Values in parentheses are percentages unless otherwise indicated. *Patients with a high Brinkman index (1000 or higher) at the time of initial resection of superficial oesophageal cancer; †patients who drink alcohol on more than 5 days per week; ‡χ^2 test. n.s., Not significant cancer between patients with SOPCs and/or subsequent multiple oesophageal cancers and those without. Among 43 patients with SOPC, nine had died by the census date, six from causes related to SOPC, two from non-malignant causes and one from a cause related to recurrence of antecedent other primary cancer.

Discussion

In the present study, 43 patients (12 per cent) developed SOPC and the 5-year cumulative occurrence rate was 15 per cent. About half occurred in the stomach or hypopharynx, both organs observable by upper gastrointestinal endoscopy. Therefore, the role of upper gastrointestinal endoscopy in the diagnosis of SOPC is expected to increase with the number of patients showing prolonged survival after treatment of superficial oesophageal cancer. On the other hand, subsequent multiple oesophageal cancers were found in 19 patients (5 per cent) and the 5-year cumulative occurrence rate was 7 per cent. It is well known that the occurrence of synchronous multiple oesophageal cancers is common in patients with oesophageal cancer. The incidence of subsequent multiple oesophageal cancers may increase as the use of endoscopic resection becomes more widespread. Furthermore, of the 267 patients who underwent surgical resection, five (2 per cent) had subsequent multiple oesophageal cancers in the residual cervical oesophagus. Therefore, careful observation of the residual oesophagus by regular endoscopy is essential after both endoscopic and surgical resection.

In the present study, subsequent cancers of the stomach and hypopharynx developed significantly more frequently in patients who were heavy smokers. Similarly, the incidence of multiple primary cancers is known to be significantly higher in heavy smokers[8,18]. It has been reported that heavy drinkers are at risk of developing multiple oesophageal tumours after endoscopic resection for superficial oesophageal cancer[19]. Although the present study did not confirm this observation, further investigation of risk factors from various aspects is necessary.

When following patients after treatment of oesophageal cancer, upper gastrointestinal endoscopy is done every 6 months after endoscopic mucosal resection and every 12 months after surgical resection. This regular examination has permitted detection of subsequent gastric cancers at an early stage; all of these have been treated by complete resection. In addition, all hypopharyngeal cancers found incidentally by regular endoscopic examination have shown a favourable clinical course. In all cases of subsequent multiple oesophageal cancer, the new lesions were also superficial. These findings confirm the value of the postoperative interval employed for endoscopic examination.

Among patients who had postoperative subsequent multiple cancers, the 5-year cumulative occurrence rate of upper gastrointestinal cancer detectable by endoscopy was 15 per cent. Gastric cancer, hypopharyngeal cancer and subsequent oesophageal cancer have a high likelihood of cure by less invasive local treatments such as endoscopic resection and localized radiation therapy[20,21].

Many previous researchers have observed no significant difference in survival between patients with multiple other primary cancers associated with oesophageal cancer and those without[5,6,8,9,22]. The present investigation confirmed this observation. However, six of nine deaths among the 43 patients with SOPC were due to that tumour. Thus, subsequent cancer may become a major cause of death among long-term survivors after cured oesophageal cancer as the number of such patients increases.

In conclusion, gastric cancer and hypopharyngeal cancer appear to occur frequently after resection of superficial oesophageal cancer. The possible risk factors for SOPC include a history of heavy smoking at the time of initial resection. Follow-up observation by upper gastrointestinal endoscopy should become more important for patients after treatment of oesophageal cancer.

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Original article

Evaluation of the necessity for gastrectomy with lymph node dissection for patients with submucosal invasive gastric cancer

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Background: When cancer cells are found in the submucosal layer of an endoscopically resected specimen, patients are recommended to undergo gastrectomy with lymph node dissection. If it were possible to identify those patients in whom the risk of lymph node metastasis was negligible, it might be possible to avoid surgery.

Methods: Among those who underwent gastrectomy for gastric cancer from 1980 to 1999, 1091 patients with a cancer invading the submucosa were studied. Clinicopathological factors (sex, age, tumour location, macroscopic type, size, ulceration, histological type, lymphatic–vascular involvement and degree of submucosal penetration) were investigated for their possible association with lymph node metastasis.

Results: Lymph node metastases were found in 222 patients (20·3 per cent). Univariate analysis showed that larger tumour size (more than 30 mm), undifferentiated histological type, lymphatic–vascular involvement and massive submucosal penetration had a significant association with lymph node metastasis. Tumour size, histological type and lymphatic–vascular involvement were independent risk factors for lymph node metastasis. By combining these three factors with submucosal penetration of less than 500 µm, 117 patients could be selected as having a minimal risk of lymph node metastasis (95 per cent confidence interval 0–3·1 per cent).

Conclusion: Lymphadenectomy may not be necessary for patients with gastric cancer invading the submucosa who fulfil the above conditions

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Introduction

Early gastric cancer (EGC) is defined as cancer in which tumour invasion is confined to the mucosa or submucosa regardless of the presence of regional lymph node metastasis[1]. For intramucosal cancer, it has already been noted that lesions with a differentiated histological type and without lymphatic–vascular involvement or ulcerative findings within the cancerous lesion have a minimal risk of lymph node metastasis[2]. Recently, local treatment for intramucosal cancer by endoscopic resection has been generally accepted as an adequate therapeutic method in Japan. In patients with submucosal invasive cancer, gastrectomy with complete removal of first- and second-tier lymph nodes has been performed as the standard treatment[3–6], because the incidence of lymph node metastasis is approximately 20 per cent[4,7–14]. If a group of patients with submucosal invasive cancer and a negligible risk of lymph node metastasis can be defined, it may be possible to modify this therapeutic strategy, possibly avoiding the risks of gastrectomy and the effects of this operation on the patient's quality of life[15–20]. Although the relationship between lymph node metastasis and the depth of submucosal invasion has been reported recently[12,21], the number of patients was too small to show statistical significance. The purpose of this study was to evaluate whether local treatment for submucosal invasive cancer might be acceptable using data from a large number of patients from a single institution.

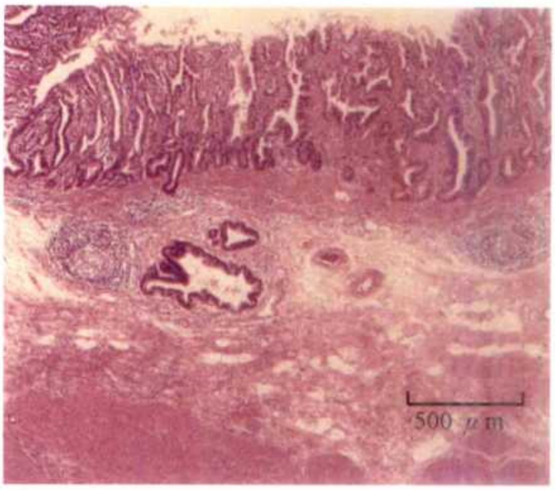
Patients and methods

Among those who underwent gastrectomy for gastric cancer from 1980 to 1999, 1091 patients with a cancer invading the submucosa were studied. Nine clinicopathological factors were evaluated by means of univariate analysis for their possible association with lymph node metastasis. Furthermore, multivariate analysis was performed using a logistic model with a stepwise method. The factors analysed, according to the Japanese classification of gastric carcinoma[1], were sex, age, tumour location, macroscopic type, size, presence or absence of peptic ulceration, the two major histological types (differentiated or undifferentiated type), lymphatic–vascular involvement and degree of submucosal penetration. Histologically, well and moderately differentiated tubular adenocarcinoma and papillary adenocarcinoma were classified as differentiated histological type; poorly differentiated adenocarcinoma and signet-ring cell carcinoma were classified as undifferentiated histological type[22]. The presence of a peptic ulcer or peptic ulcer scar (defined endoscopically as converging folds and histologically as a deformity of the muscularis propria or fibrosis in the submucosal or deeper layer) within a cancerous lesion was defined as a positive ulcerative finding. The depth of invasion into the submucosal layer was measured at the deepest level of penetration of the cancer cells with an ocular lens scale (*Fig. 1*). The degree of submucosal penetration was classified as SM1 (penetration of submucosal layer less than 500 µm from the muscularis mucosa) or SM2 (500 µm or more).

Statistical analysis was carried out using the SAS program (SAS Institute, Cary, North Carolina, USA). The association of lymph node metastasis with clinicopathological variables was assessed using a simple χ^2 test. $P < 0.05$ was considered significant. The probability of lymph node metastasis was estimated with 95 per cent confidence intervals (c.i.).

Results

The mean age of the patients with cancer invading the submucosa was 59·4 years. The mean tumour size was 33·4 mm and the incidence of lymph node metastasis was 20·3 per cent. The presence of lymph node metastasis had a



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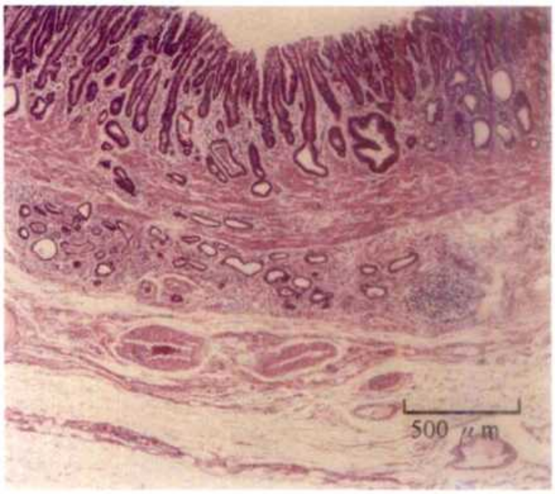

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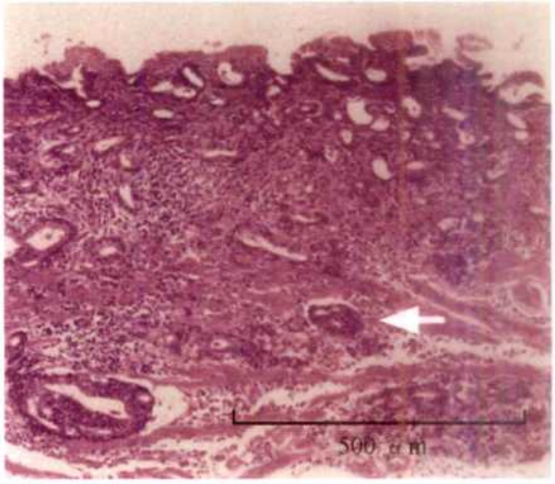

c SMI plus lymphovascular invasion

Fig. 1 Estimation of submucosal invasion measured with an ocular lens scale. In **a** and **b** the degree of submucosal penetration was classified as SM1 (less than 500 µm from the muscularis mucosa) (haematoxylin and eosin stain, original magnification × 4). In **c**, although the depth of invasion into the submucosal layer measured at the deepest penetration of the cancer cells was classified as SM1, lymphatic–vascular involvement was found (white arrow) (haematoxylin and eosin stain, original magnification × 10)

significant correlation with tumour size larger than 30 mm, undifferentiated histological type, lymphatic–vascular involvement and submucosal penetration classified as SM2 (*Table 1*). These four clinicopathological factors were assessed by multivariate analysis using a logistic model with a stepwise method (*Table 2*). Independent risk factors for lymph node metastasis were presence of lymphatic–vascular involvement, undifferentiated histological type and tumour size larger than 30 mm in diameter. The incidence of lymph node metastasis of submucosal invasive cancer that was negative for these three risk factors was 5·6 (95 per cent c.i. 3·1–9·0) per cent (15 of 270 patients). Furthermore, when these three risk factors were absent, and there was only minimal submucosal penetration (SM1), the incidence of lymph node metastasis was zero (95 per cent c.i. 0–3·1 per cent) (none of 117 patients).

Table 1 Relationship between clinicopathological factors and lymph node metastasis of submucosal invasive cancer, and results of univariate analysis

	No. of patients	Lymph node metastasis		*P*
		No	Yes	
Sex				0·221
Male	761	613	148 (19·4)	
Female	330	256	74 (22·4)	
Age (years)				0·198
< 59	484	377	107 (22·1)	
≥ 59	607	492	115 (18·9)	
Location in stomach				0·100
Upper third	159	136	23 (14·5)	
Middle third	575	457	118 (20·5)	
Lower third	357	276	81 (22·7)	
Macroscopic type				0·091
Raised	285	216	69 (24·2)	
Depressed	806	653	153 (19·0)	
Tumour size (mm)				< 0·001
≤ 30	602	510	92 (15·3)	
> 30	489	359	130 (26·6)	
Ulcer findings				0·797
No	523	418	105 (20·1)	
Yes	568	451	117 (20·6)	
Histological type*				0·002
Differentiated	683	563	120 (17·6)	
Undifferentiated	408	306	102 (25·0)	
Lymphatic–vascular involvement				< 0·001
No	703	641	62 (8·8)	
Yes	388	228	160 (41·2)	
Degree of submucosal penetration				< 0·001
SM1	296	267	29 (9·8)	
SM2	795	602	193 (24·3)	

Values in parentheses are percentages. *Differentiated type includes papillary and tubular adenocarcinoma; poorly differentiated adenocarcinoma and signet-ring cell carcinoma are classified as undifferentiated type

Table 2 Independent risk factors for lymph node metastasis from submucosal invasive cancer

	Relative risk	Standard error	*P*
Lymphatic–vascular involvement (absence *versus* presence)	6·422	0·176	< 0·001
Histological type (differentiated *versus* undifferentiated)	1·752	0·172	0·001
Tumour size (≤ 30 *versus* > 30 mm)	1·569	0·170	0·008

Discussion

The presence of lymph node metastasis is the most important prognostic factor for patients with EGC[3,23,24]. Radical surgery with lymph node dissection has provided an excellent therapeutic outcome; the 5-year survival rate after curative resection is more than 90 per cent including recent European series[3,25–29]. Local tumour resection without lymph node dissection can be employed for some EGCs, but the indications for this should be considered carefully and a detailed evaluation of a large number of cases is required.

Endoscopic mucosal resection (EMR) for selected intramucosal EGCs, for which the possibility of lymph node metastasis is almost zero, has been widely accepted as a curative therapeutic strategy in Japan. Patients with submucosal invasive cancer, on the other hand, are recommended to undergo surgical gastrectomy with regional lymph node dissection. In this institution the survival rate in these patients, excluding non-cancer deaths, was 96 per cent at 5 years after radical surgery[27]. However, considering the negative effect of gastrectomy on the quality of life[15–20], further subclassification of submucosal invasive cancer to select those with a minimal risk of lymph node metastasis would be beneficial. Several authors have suggested that tumours with a maximum depth of invasion of less than 300 µm have a limited risk of lymph node metastasis[12,21,30–33], but the numbers of patients analysed were too small to give a meaningful upper limit of the 95 per cent c.i.

The present study analysed the risk factors for lymph node metastasis from submucosal invasive cancer using a large number of patients treated in a single institution to identify lesions that were curable by local treatment. Among differentiated tumours smaller than 30 mm without lymphatic–vascular involvement, the incidence of lymph node metastasis was 5·6 (95 per cent c.i. 3·1–9·0) per cent. This figure is not acceptable for good-risk Japanese patients whose operative mortality and 5-year survival rates are excellent. When the degree of submucosal penetration was added to above factors, no lymph node metastasis (95 per cent c.i. 0–3·1 per cent) was seen in patients with SM1 grade of invasion in the absence of the three independent risk factors.

These criteria are based on complete histological examination of the resected specimen. Some factors, especially lack of lymphatic–vascular involvement or SM1 grade, which are associated with a minimal risk of lymph node metastasis, are available only after histological examination of the resected specimen. Therefore it is of paramount importance that the specimen is resected endoscopically such that accurate histological examination can be carried out. When the wider application of local treatment for patients with such submucosal invasive cancer is considered, it is essential to evaluate the resected specimen accurately and decide whether an additional surgical procedure is warranted. Korenaga *et al.*[34] reported that correct assessment of the depth of submucosal invasion in an endoscopically resected specimen was difficult when the size of tumour exceeded 15 mm. This is because such lesions are often resected in multiple fragments. A new technique of endoscopic resection using an insulation-tipped electrosurgical knife to remove a large lesion *en bloc* has been developed[35]. If resected *en bloc*, lesions larger than 15 mm can be examined as accurately as surgically resected material. This enables EMR to be offered to many patients who would otherwise have been treated by surgical intervention.

The endoscopic distinction between mucosal and submucosal invasion is made correctly in only approximately 80 per cent of tumours[36,37], which means that the pretreatment diagnosis would be incorrect for 20 per cent of the tumours that are candidates for local treatment.

In conclusion, additional surgery with lymph node dissection is of little benefit for tumours that satisfy the following four conditions: (1) no lymphatic–vascular involvement, (2) tumour size smaller than 30 mm, (3) differentiated histological type and (4) depth of submucosal penetration classified as SM1.

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Original article

Local heat-shock priming-induced improvement in microvascular perfusion in osteomyocutaneous flaps is mediated by heat-shock protein 32

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Background: Stress conditioning is thought to improve microvascular free flap survival but the mechanisms of protection are not clear. The aim of this study was to determine whether local induction of heat-shock protein (HSP) 32 improves microvascular perfusion in transferred osteomyocutaneous flaps.

Methods: The hindlimb harvest region of osteomyocutaneous flaps in Wistar rats was subjected to stress conditioning by local heating (30 min, 42·5°C) 24 h before microvascular flap transfer. In a second group of animals, after heat-shock priming, the action of HSP-32 was inhibited by tin protoporphyrin IX. Animals with unconditioned flaps served as controls. After transfer, the microcirculation of the muscle, cutaneous, subcutaneous and periosteal tissue of the flap was analysed quantitatively for 6 h using intravital fluorescence microscopy.

Results: Immunohistochemistry revealed that HSP-32 was detectable only after priming and not in unconditioned flaps. Priming did not alter functional capillary density or capillary red blood cell velocity compared with that in unconditioned flaps. However, heat-shock priming induced significant capillary dilatation ($P < 0{\cdot}05$) and thus a substantial increase in capillary blood flow volume ($P < 0{\cdot}05$) in all tissues of the transferred flaps. Inhibition of HSP-32 by tin protoporphyrin IX completely abolished the priming-induced improvement in capillary perfusion, as indicated by the lack of increased capillary diameters and volumetric blood flow.

Conclusion: The present study demonstrated that stress conditioning by local heat-shock priming improves nutritive perfusion in osteomyocutaneous flaps by capillary dilatation, probably mediated through the vasoactive action of HSP-32.

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Introduction

Microvascular transfer of composite flaps has become well accepted in reconstructive surgery[1]. However, vasoconstriction and capillary perfusion failure within the flap microcirculation are frequent complications and might be responsible for the loss of flap viability[2].

Improvement of flap viability has been demonstrated using stress conditioning by heat shock and recovery in isolated pedicled skin flaps[3]. Cells exposed to supraphysiological levels of heat respond by transiently decreasing the production of most cellular polypeptides and rapidly synthesizing a number of proteins that have been remarkably conserved during evolution and are known as heat-shock proteins (HSPs)[4]. According to their molecular mass and degree of homology within nucleotide and amino acid sequences, HSPs have been classified into distinct families. Several HSPs of the 70-kDa family are known to prevent misfolding of newly synthesized proteins and escort proteins targeted for other cellular compartments[5]. HSP-32, which belongs to the family of small HSPs, has been identified[6] as haem oxygenase 1, and has been shown to be the rate-limiting enzyme in the catabolism of haem to biliverdin, free iron and carbon monoxide. Finally, carbon monoxide is known to act as a potent vasodilator[7].

Which mechanisms of action of these different HSPs proteins are involved in improving the viability of transferred flaps is not yet clear. The objective of this study was to determine whether local heat-shock priming protects microvascular perfusion of osteomyocutaneous flaps and, if so, whether this involves the action of HSPs of the 70-kDa family or distinctly the vasoactive properties of HSP-32.

Materials and methods

Animals

Twenty-four Wistar (WI dx dx/PH) rats with a body-weight ranging from 270 to 340 g were used. The inbred strain WI dx dx/PH is homozygous for the autosomal recessive gene *dx*, and was used to avoid an anaphylactic reaction upon application of dextran. All animals were housed one per cage and had free access to tap water and standard pellet food (Altromin, Lage, Germany). The study complied with the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals and was approved by the local animal care committee.

Surgical procedure

The surgical procedure for the preparation of peripheral tissues was similar to that described previously in detail[8]. In brief, the animals were anaesthetized with intraperitoneal pentobarbital sodium 50 mg per kg body-weight (Abbott, Chicago, Illinois, USA), tracheotomized to facilitate breathing, and then placed on a heating pad, which maintained body temperature between 36 and 37°C. Polyethylene catheters were inserted into the right carotid artery and left jugular vein to allow monitoring of blood pressure, infusion of isotonic saline (1 ml per 100 g body-weight per h) and injection of fluorescent dyes for intravital microscopy.

The left hindlimb was shaved and epilated. A circular skin island (15 mm in diameter) was incised circumferentially down to the underlying muscle fascia. After exposing and cutting the distal part of the tibia with wire-cutting forceps proximal to the tibiofibular junction, the gracilis anterior, gracilis posterior and semitendinosus muscles were dissected medioproximally. The proximal part of the tibia was then exposed and cut similarly to the distal end. The femoral vessels were exposed by an additional linear skin incision from the skin island up to the inguinal crease and subsequently dissected by ligating and dividing the popliteal, genicular, muscular and superficial epigastric branches. Thus, the harvest of the osteomyocutaneous flap, consisting of the tibial bone segment and attached periosteum, the gracilis and semitendinosus muscles, as well as the overlying skin and subcutaneous tissue was completed. The osteomyocutaneous flap was finally transferred to the contralateral hindlimb by end-to-end anastomoses to the right femoral vessels using a conventional technique of seven to ten simple interrupted 11/0 nylon sutures.

Intravital fluorescence microscopy

After transfer, the tissues were exposed on a micromanipulator-adjusted stage placed close to the right groin, or the left groin in controls. Epi-illumination microscopy was performed with a modified Axiotech microscope (Zeiss, Jena, Germany), which included a 100-W mercury arc-lamp and a blue filter combination (450–490 nm excitation; more than 520 nm emission wavelength) for visualizing green fluorescence[9,10]. The microcirculation of muscle, skin, subcutaneous tissue and periosteum was analysed after intravenous injection of 0·25 ml 5 per cent fluorescein isothiocyanate-labelled dextran (molecular weight 150 000 Da; Sigma, St Louis, Missouri, USA). The microscopic images were recorded by a charge-coupled device video camera (FK6990; COHU, San Diego, California, USA) and were transferred to a video system (S-VHS Panasonic 7350; Matsushita, Tokyo, Japan).

Video analysis

Microcirculatory parameters were analysed off-line using computer-assisted image analysis systems, Capimage (Zeintl, Heidelberg, Germany) and Capiflow (Capiflow, Kista, Sweden). Analysis included the determination of functional capillary density (defined as the total length of capillaries with blood flow per unit area of observed tissue (per cm)), capillary red blood cell velocity (mm/s) and capillary diameter (µm). Capillary blood flow (CBF; pl/s) was calculated from red blood cell velocity (V_{RBC}) and diameter (D) for each vessel as $CBF = \pi \times (D/2)^2 \times V_{RBC}$.

Stress conditioning

Local heat-shock priming was induced by heating the left hindlimb of the anaesthetized animal in a water bath 24 h before flap transfer. The heating procedure was standardized by increasing the temperature in muscle to 42·5°C for 30 min. Thereafter, animals were allowed to recover with free access to standard pellet food and tap water until harvest of the osteomyocutaneous flaps.

Immunohistochemical staining

All chemicals were purchased from Sigma–Aldrich (Deisenhofen, Germany) unless specified otherwise.

Formalin-fixed, paraffin-embedded sections of cutaneous, subcutaneous and muscle tissue were used to stain HSP-32 and proteins of the 70-kDa HSP family. After antigen retrieval using microwave irradiation[11] and blockade of endogeneous peroxidase activity, tissue sections were incubated at 37°C for 1 h with either the primary anti-HSP-32 antibody (dilution 1:200; StressGen Biotechnologies, Sidney, British Columbia, Canada) or the primary anti-HSP-70 antibody that recognized both HSP-72 and HSP-73 (dilution 1:25; Dako, Carinteria, California, USA). As secondary antibody, a biotinylated goat antirabbit antibody was used for streptavidin–biotin complex peroxidase staining. 3,3′-Diaminobenzidine and 3 per cent nickel chloride were used as chromogens. Slides were left unstained or were counterstained with haematoxylin. As a negative control, slides were treated similarly but without the primary antibody.

Semiquantitative analysis of the staining intensity was performed blind according to the procedure described by Page *et al.*[12]. Briefly, a 0–4 score was applied, depending on the intensity and extent of colour: 0, negative (comparable to control sections); 1, very weakly positive; 2, weakly positive; 3, positive; and 4, strongly positive.

Experimental groups

The 24 animals were divided into three groups. In group 2 (*n* = 8) and group 3 (*n* = 8) stress conditioning by local heat-shock priming was induced 24 h before transfer of the osteomyocutaneous flaps. Animals in group 3 were additionally treated with intraperitoneal tin protoporphyrin IX 50 µmol per kg body-weight (Porphyrin Products, Logan, Utah, USA), a competitive inhibitor of HSP-32, at the time of heat-shock priming. Osteomyocutaneous flaps without conditioning before transfer served as controls (group 1; *n* = 8). Microcirculatory analyses of muscle, skin, subcutaneous tissue and periosteum were performed at 2, 4 and 6 h after transfer of the osteomyocutaneous flaps. Tissue samples for immunohistochemistry were obtained after the 6-h intravital microscopic procedure.

Statistical analysis

Results were expressed as mean(s.e.m.). Analysis of normality of distribution and equal variance was performed first. Then one-way analysis of variance or Kruskal–Wallis analysis of variance was performed, followed by appropriate *post hoc* comparisons. Statistical significance was set at $P < 0.05$.

Results

Surgical procedure and systemic circulatory variables

The time required for harvest (range 150–210 min) and transfer (range 57–76 min) of the osteomyocutaneous flaps did not differ between the three groups, indicating comparable levels of surgical trauma and ischaemia to the tissues under investigation. During preparation and microscopy, assessment of systemic haemodynamics revealed physiological values with no significant differences in mean arterial blood pressure, which ranged from 94 to 133 mmHg.

Immunohistochemistry

HSPs of the 70-kDa family and HSP-32 had a comparable expression pattern among the cell types. Positive staining suggested predominant expression of HSP-32, as well as HSPs of the 70-kDa family, in the cytoplasm of endothelial lining cells compared with epithelial cells, subcutaneous fat cells and striated muscle cells. In *Fig. 1* this specific expression pattern is demonstrated for HSP-32 in striated muscle. To assess expression of HSPs semiquantitatively, endothelial linings in three slices of skin, subcutaneous tissue and muscle were analysed in each of four osteomyocutaneous flaps per group. In transferred osteomyocutaneous flaps without stress conditioning immunohistochemical staining demonstrated a distinct expression of HSPs of the 70-kDa family (*Table 1*). This pattern of expression was not affected substantially by stress conditioning (*Table 1*). In contrast, HSP-32 was not detectable in unconditioned flaps, whereas preconditioning resulted in a marked expression of HSP-32 (*Fig. 1* and *Table 1*). Additional application of tin protoporphyrin IX did not alter the expression pattern of HSP-32.

Tissue-confined microvascular perfusion in transferred osteomyocutaneous flaps

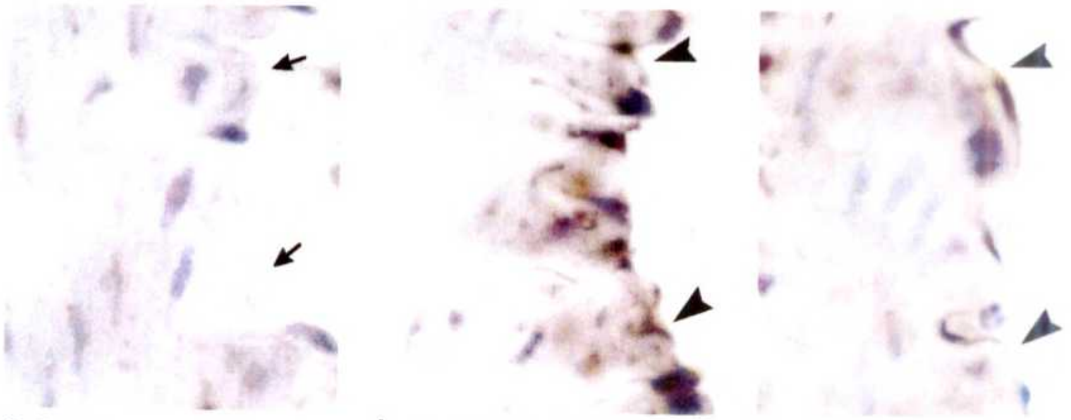
While capillaries were arranged in parallel to each other in skeletal muscle and in a mesh-like pattern in subcutaneous tissue and skin, two distinct types of capillary arrangement were found in periosteum. Adjacent to the major supplying and draining vessels, which originate from the saphenous vessels, the capillaries were arranged as densely meshed networks of microvessels. In contrast, capillaries distant from the supplying and draining vessels of the periosteum were straightened in parallel to the longitudinal axis of the tibial bone.

The functional capillary density of the muscle, periosteum and subcutaneous region of the osteomyocutaneous flaps did not change significantly during the 6-h time course of reperfusion after flap transfer, regardless of whether heat-shock priming was performed. In addition, functional capillary density was similar after local heat-shock priming to that in controls. Neither was it influenced significantly by the inhibition of HSP-32 (*Fig. 2a*). In skin, however, heat-shock priming induced a slight but significant increase in functional capillary density over the entire 6-h observation period, which was completely abolished after inhibition of HSP-32 by tin protoporphyrin IX (*Fig. 2a*).

Most strikingly, heat-shock priming resulted in marked vasodilatation, as indicated by significantly increased capillary diameter compared with that in unconditioned controls ($P < 0{\cdot}05$). This effect was observed in all tissues analysed by 2 h of reperfusion (data not shown), and was still evident at 6 h after flap transfer (*Fig. 2b*). Although capillary red blood cell velocity remained essentially unchanged after local heat-shock priming (*Fig. 2c*), the overall capillary volumetric blood flow was significantly increased at 2, 4 and 6 h after transfer compared with that of unconditioned controls ($P < 0{\cdot}05$). This again included all tissues analysed, i.e. muscle, periosteum, subcutaneous tissue and skin (*Fig. 2d*). Inhibition of HSP-32 with tin protoporphyrin IX before transfer completely abolished the priming-induced dilatation of capillaries and, in parallel, abrogated the increase in volumetric capillary blood flow after 2, 4 and 6 h of reperfusion (*Figs 2b,d*).

Discussion

Microvascular free tissue transfer has replaced classical flap techniques in many cases and become a well accepted reconstructive technique for coverage of large tissue defects resulting from trauma or ablative surgery. Improved



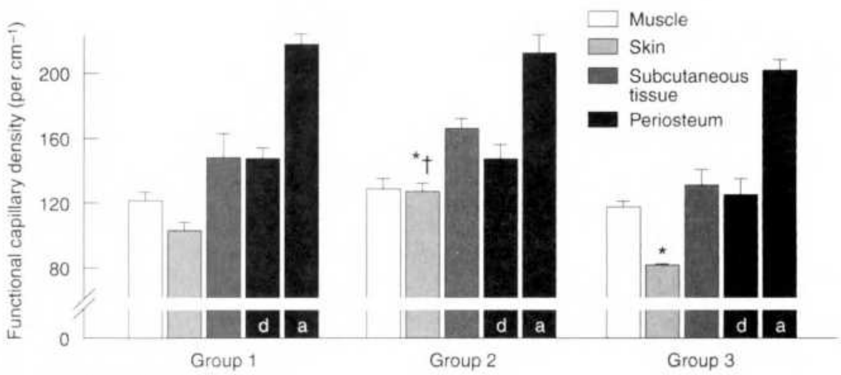
a Control **b** After stress conditioning **c** After inhibition

Fig. 1 Immunohistochemical expression pattern of heat-shock protein (HSP) 32 in arterioles of striated muscle of transferred osteomyocutaneous flaps **a** without stress conditioning, **b** after stress conditioning and **c** after stress conditioning with additional inhibition of HSP-32. After stress conditioning HSP-32 was predominantly expressed in the cytoplasm of endothelial lining cells (**b** and **c**, arrow heads) compared with unstressed controls (**a**, arrows)

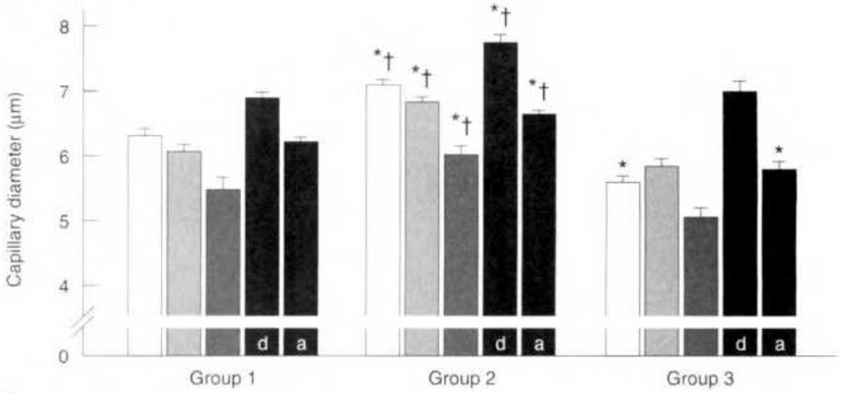
Table 1 Expression of heat-shock proteins of the 70-kDa family and heat-shock protein 32

	HSPs of 70-kDa family			HSP-32		
	Muscle	Subcutaneous tissue	Skin	Muscle	Subcutaneous tissue	Skin
Group 1	3·7(0·1)	3·8(0·2)	3·8(0·2)	0·0(0·0)	0·0(0·0)	0·0(0·0)
Group 2	3·7(0·3)	3·6(0·2)	3·7(0·1)	3·8(0·1)*	3·7(0·1)*	3·8(0·2)*
Group 3	3·6(0·2)	3·7(0·2)	3·7(0·3)	3·7(0·2)*	3·8(0·2)*	3·9(0·2)*

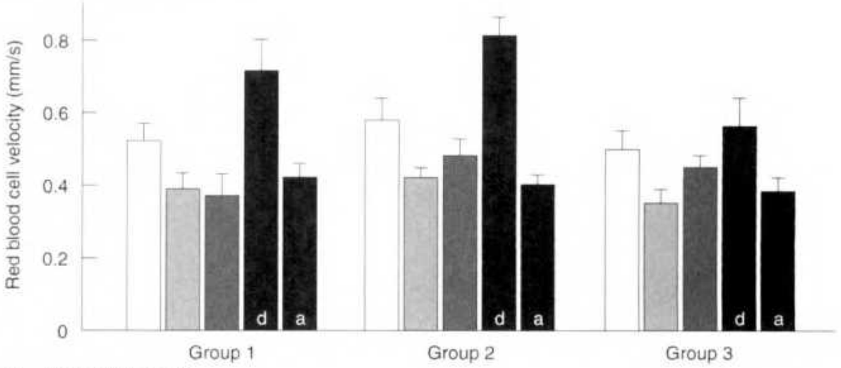
Values are mean(s.e.m.) arbitrary units calculated by semiquantitative analysis of the intensity and extent of immunohistochemical labelling. In group 2 stress conditioning and in group 3 stress conditioning and inhibition of heat-shock protein (HSP) 32 was performed 24 h before flap transfer. Animals with unconditioned osteomyocutaneous flaps served as controls (group 1). *$P < 0{\cdot}05$ *versus* group 1 (Student–Newman–Keuls test)



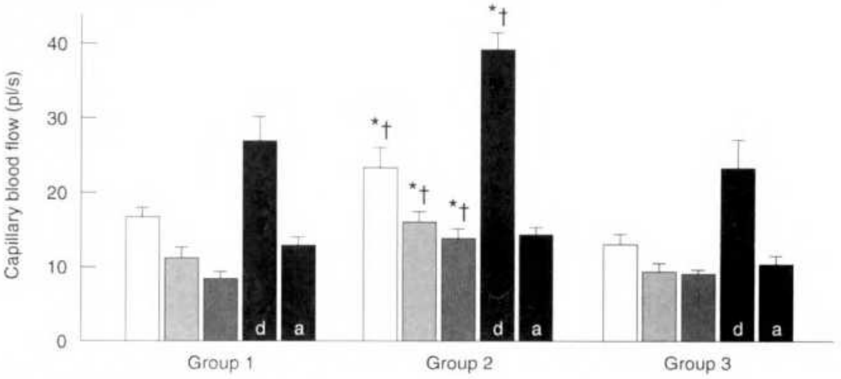
a Capillary density



b Capillary diameter



c Red cell velocity



d Volumetric flow

Fig. 2 **a** Functional capillary density, **b** capillary diameter, **c** capillary red blood cell velocity and **d** capillary volumetric blood flow in muscle, skin, subcutaneous tissue and periosteum of osteomyocutaneous flaps at 6 h after microvascular transfer. In group 2, flaps were subjected to stress conditioning by local heat-shock priming 24 h before transfer. Animals in group 3 underwent stress conditioning and, additionally, inhibition of heat-shock protein 32 by tin protoporphyrin IX. Osteomyocutaneous flaps without stress conditioning served as controls (group 1). In periosteum, regions of analysis were differentiated as being located adjacent (a) or distant (d) from the major supplying and draining vessels. Values are mean(s.e.m.). $^*P < 0{\cdot}05$ *versus* group 1, $^\dagger P < 0{\cdot}05$ *versus* group 3 (Student–Newman–Keuls test) appearance and function through reconstruction of skeletal integrity are achieved using osteomyocutaneous flaps[13]. Nevertheless, the rate of failure of microvascular free flaps documented in the literature amounts to 10 per cent, and may be higher depending on the type of flap used[14]. Thrombosis at the anastomotic site is the most common cause of failure of a microvascular free flap[14,15]. However, flap loss is also observed with a patent anastomosis[16,17]. Under these circumstances flap loss is thought to be due to microcirculatory failure. Because the vast majority of research has concentrated on thrombotic occlusion of the surgically repaired pedicle vessels, little is known about the pathophysiology of the nutritional vessels of revascularized tissue. Apart from wound infection and showering of microemboli downstream from the arterial anastomosis, microvascular vasoconstriction is predominantly thought to be responsible for impairment of nutritive microcirculation in freely transferred flaps[15,17,18].

Several attempts have been made to improve flap survival by antagonizing microvascular constriction using different exogenous vasodilators including prostacyclin[19,20] and L-arginine[21]. In contrast, the present study demonstrated sustained capillary dilatation and thus improved microvascular perfusion by enhancement of endogenous vasodilator properties as a result of heat-shock priming in transferred osteomyocutaneous flaps.

Induction of a heat-shock response requires an increase in the temperature of the tissue to 42·5°C. Whole-body hyperthermia may not be feasible because a body temperature of 42·5°C may critically affect systemic organ functions. In contrast, local heating allows application of this temperature for preconditioning, inducing a significant stress response in tissue to be grafted 24 h later, without affecting systemic homoeostasis, core temperature or provoking a distant organ stress response. The lack of systemic stress response was verified by analysis of tissue samples of the contralateral hindlimb and the liver, taken at the end of the 6-h observation period; there was no expression of HSP-32 in these tissues.

Data from the present study show that the rise in volumetric blood flow in individual capillaries was predominantly due to capillary dilatation, because capillary red blood cell velocity remained essentially unaffected by local heat-shock priming. As a result, nutritive perfusion of the muscle, skin, subcutaneous tissue and periosteum of osteomyocutaneous flaps was improved by local heat-shock priming, at least for several hours after the transfer procedure.

A limitation of the study is that the model used is acute and did not allow microcirculatory studies for more than a few hours after transfer. However, in another study performed in skin flaps in mice, it was demonstrated that expression of HSP-32, as well as improvement in microvascular perfusion, lasted for up to 6 days after stress conditioning[22].

The present study did not include a control group with pretransfer anaesthesia and immersion of the hindlimb at normothermia. However, in pilot experiments, which were done to establish the model for local heat-shock response, a local HSP-32 response was not detected in the hindlimb following anaesthesia alone, or local heating with temperatures below 41·5°C. Therefore, the experimental design of this study did not include an additional control group with pretransfer anaesthesia and immersion of the hindlimb at normothermia. The control group with no pretransfer manipulation was chosen in order to simulate more closely the clinical conditions for studying heat-shock treatment.

Heat-shock priming is characterized by a transient decrease in the production of most cellular polypeptides, and rapid transcription and subsequent translation of a set of highly conserved HSPs[23]. HSPs, in particular those of the 70-kDa family, are proposed to improve microvascular free flap survival. The 70-kDa HSP family includes the inducible HSP-72 as well as the constitutively expressed HSP-73[23]. With respect to the present data, a contribution from the 70-kDa HSP family to capillary dilatation after transfer seems to be unlikely, because expression of HSPs of the 70-kDa family could be detected in comparable amounts in both conditioned and unconditioned osteomyocutaneous flaps. In contrast, expression of HSP-32 was evident only after stress conditioning; this protein could not be detected in any of the tissues from unconditioned flaps. Moreover, application of tin protoporphyrin IX at a dose that is reported to inhibit the activity of HSP-32 for up to 72 h[24] completely abolished the heat shock-induced improvement in microvascular perfusion after flap transfer. Thus, the present data strongly suggest that HSP-32 is crucially involved in the local heat shock priming-induced capillary dilatation.

HSP-32, which has been identified as haem oxygenase 1, is the rate-limiting enzyme in the catabolism of haem to biliverdin, free iron and carbon monoxide[25]. Carbon monoxide, a gaseous monoxide, is known to induce vasorelaxation analogous to nitric oxide[26]. However, there is strong evidence that vasodilatation following heat shock may not be mediated by nitric oxide[27]. Therefore, to explain the observed dilatation of capillaries dependent on heat-shock priming, two distinct physiological mechanisms could be proposed. Carbon monoxide has been shown to induce relaxation of vascular smooth muscle cells causing arteriolar vasodilatation[26]. As a result of the increased blood volume reaching the nutritive microvasculature following arteriolar relaxation, capillaries may dilate passively. On the other hand, relaxation of fat-storing Ito cells, the liver's specific microvascular pericytes covering the sinusoidal wall, mediated by carbon monoxide can modulate vascular tone[28]. Consequently, the observed dilatation of capillaries may also result from direct pericyte relaxation. Therefore, the observed improvement in microvascular perfusion in osteomyocutaneous flaps after transfer by local heat-shock priming may result mainly from the vasodilator property of HSP-32. Thus, induction of HSP-32 may be able to preserve overall tissue function after flap transfer, not only by the well known antioxidative action of bilirubin, which has been shown effectively to reduce oxidant-mediated leucocyte responses[29] and postischaemic organ dysfunction[30], but also by the vasoactive action of carbon monoxide, improving nutritional blood supply.

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Original article

Extensive surgical cytoreduction and intraoperative hyperthermic intraperitoneal chemotherapy in patients with pseudomyxoma peritonei

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Background: Pseudomyxoma peritonei remains a fatal disease. However, extensive surgical cytoreduction combined with intraoperative heated intraperitoneal chemotherapy (HIPEC) has recently emerged as a new treatment modality, which might improve survival.
Methods: Patients underwent treatment if the tumour appeared to be technically resectable on preoperative abdominal computed tomography and there were no distant metastases. After aggressive surgical cytoreduction, HIPEC with the administration of mitomycin C was performed for 90 min. Depending on histological grading, patients received adjuvant 5-fluorouracil and leucovorin therapy.
Results: Forty-six patients were treated. Optimal surgical cytoreduction was obtained in 40 patients. Postoperative surgical complications occurred in 18 patients. Four patients died as a direct result of the treatment. Bone marrow suppression due to mitomycin C toxicity occurred in 22 patients. There was no other major toxicity related to the HIPEC procedure. After a median follow-up of 12 months, 40 patients are alive, eight of whom have proven recurrence. The actuarial survival rate (Kaplan–Meier) at 3 years was 81 per cent.
Conclusion: These results confirm that extensive surgery combined with HIPEC is feasible in patients with pseudomyxoma peritonei and that improved long-term survival might be achieved.

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Introduction

Pseudomyxoma peritonei is a rare condition characterized by intra-abdominal extracellular gelatinous fluid collections and non-invasive mucinous implants on the peritoneum containing mucus-producing epithelium[1,2]. The recent development of immunohistochemical techniques has led to a better understanding of the aetiology and pathogenesis of this disease[3–5]. It is now generally accepted that pseudomyxoma peritonei is a result of neoplastic mucus-secreting cells within the peritoneal cavity that have low-grade, relatively benign, cytological features and are sparsely distributed within the extracellular mucinous collections[1]. In most cases these cells derive from a ruptured appendiceal cyst, benign adenoma or a low-grade appendiceal adenocarcinoma. It is thought that perforation of this primary process leads to the distribution of atypical cells into the abdominal cavity, the so-called redistribution phenomenon, as described by Sugarbaker[6]. Pseudomyxoma peritonei is usually diagnosed by combining the clinical findings ('jelly belly'[7]) and the histological features. The difficulty in differential diagnosis is the differentiation between pseudomyxoma peritonei and carcinosis peritonei due to well differentiated mucinous carcinoma.

Despite its relatively benign character and better understanding of its aetiology and prognostic features, the long-term survival of patients with pseudomyxoma peritonei remains poor with reported 5- and 10-year survival rates of 50 and 10–30 per cent respectively[1]. In recent decades repetitive and sometimes extended operations have usually been the sole therapeutic modality. All macroscopic tumour was removed if possible, including the appendix and in women the ovaries. Complete cure, however, could never be achieved and the disease-free interval between different surgical interventions became progressively shortened. Finally, further surgery became impossible because of extensive tumour entrapment of bowel surfaces; consequently patients eventually died from cachexia.

In the 1980s a new combined treatment option was introduced: aggressive maximal surgical cytoreduction in combination with intraoperative hyperthermic intraperitoneal chemotherapy (HIPEC)[8,9]. With this combined treatment modality a 5-year survival rate of 86 per cent was described by Sugarbaker *et al.*[10,11]. Because HIPEC in combination with surgical cytoreduction is a high-risk and demanding procedure, associated with a high complication rate, this treatment option has not gained wide application and so comparable data on its morbidity, toxicity and effectiveness in larger groups of patients with pseudomyxoma peritonei have not yet been published.

Since 1996, HIPEC in combination with extensive surgical cytoreduction has been used as a treatment schedule in patients with pseudomyxoma peritonei in this institute. This paper describes the treatment-related morbidity, disease-free interval and survival in the first series of 46 patients.

Patients and methods

Patient selection

Patients with pseudomyxoma peritonei were treated by aggressive surgical cytoreduction and HIPEC with mitomycin C (MMC) if they fulfilled the following criteria: diagnosis proven by histological or cytological examination; no sign of distant metastasis on computed tomography (CT) of the abdomen and chest; the tumour had to be technically resectable; and the patient considered medically fit to undergo major surgery. The local ethics committee approved the treatment protocol, and informed consent was obtained from all patients.

Surgical aspects

The abdomen was approached through a median incision from the xyphoid process to the pubic symphysis. Comprehensive adhesiolysis was performed. The tumour distribution was recorded according to the presence of tumour deposits in seven abdominal areas: left and right subdiaphragmatic, subhepatic, omentum/transverse colon, small intestine/mesentery, ileocaecal and pelvic. The primary tumour and/or recurrences were excised, and all visceral or parietal peritoneal surface tumour deposits were removed as completely as possible. The objective of optimal cytoreductive surgery was to leave no macroscopic tumour behind or, when this could not be achieved, only tumour deposits less than 2·5 mm in diameter. If it was impossible to peel the malignancy from the surface of an organ, the involved organ or a segment of it was excised. After bowel resection anastomoses were postponed until after the intraperitoneal MMC perfusion to prevent suture-line seeding. When parietal peritoneal surfaces were significantly involved, peritonectomy procedures, as described by Sugarbaker[12], were performed.

Perfusion

After cytoreductive surgery was completed, a Tenckhoff inflow catheter (Curl Cath™; Quinton, Bothell, Washington, USA) was introduced centrally into the abdominal cavity. Three silicone outflow drainage tubes (Dura-Sil; Biometrix, Jerusalem, Israel) were placed subphrenically on both sides and in the pelvis. Temperature sensors (Mon-a-therm™; Mallinckrodt Medical, St Louis, Missouri, USA) were attached to the catheters. The catheters were connected through a reservoir with a filter (Safe II Filtered Cardiotomy Reservoir; Polystan, Copenhagen, Denmark) to the heat exchanger (Baxter, Uden, The Netherlands) and roller pump (Polystan, Copenhagen, Denmark) and back to the patient. The skin surrounding the laparotomy wound was sutured to a retractor ring placed above the anterior surface of the abdomen. In this way expansion of the peritoneal cavity was achieved, resulting in better exposure of the entire seroperitoneal surface to the perfusate. Plastic sheeting was sutured over the laparotomy wound to prevent spillage of the perfusate and loss of heat. Centrally in the plastic cover a small hole was made to allow entrance of the surgeon's hand. The perfusion system was filled with isotonic dialysis fluid (Dianeal® PD1; Baxter). The perfusate circulated at a rate of approximately 1 l/min.

The MMC was added in three divided doses with a 30-min interval. Initially, MMC 15–40 mg/m^2 was given. In the majority of patients MMC 35 mg/m^2 was administered. At standard time intervals, samples of perfusion fluid and plasma were taken for pharmacokinetic studies. The intraperitoneal temperature was maintained between 40 and 41°C, and the perfusion lasted for 90 min. To achieve uniform temperature and drug distribution, the surgeon gently stirred the perfusate and bowel loops by hand.

After completion of the perfusion the excess fluid was drained from the abdominal cavity. Because of the expected prolonged ileus, a gastrostomy was performed for gastric decompression and a feeding tube was positioned in the proximal jejunum for early postoperative enteral nutrition. Surgical complications and toxicity attributed to MMC were recorded, according to the World Health Organization (WHO) grading for chemotherapy toxicity.

Adjuvant chemotherapy and follow-up

Based on histological findings patients were classified as having benign or malignant pseudomyxoma peritonei. Patients with malignant disease received adjuvant 5-fluorouracil (5-FU) and leucovorin if they had not previously been treated with this regimen. Some 6–12 weeks after discharge, weekly intravenous administration of 5-FU 400 mg/m^2 in bolus injection, following leucovorin 80 mg/m^2 in a 1-h infusion, was started. Adjuvant chemotherapy was continued for 6 months or until progression of the tumour was observed or intolerable toxicity occurred. Six weeks and 3 months after discharge, and then at 3-monthly intervals, patients were seen in the outpatient clinic. Physical and laboratory examination, including estimation of carcinoembryonic antigen (CEA) and CA-19.9 levels, was performed. CT of the abdomen and chest radiography were performed every 6 months, starting 3 months after operation. Time to recurrence and survival were recorded.

Results

Patients

From April 1996 until March 2000, 46 patients (19 men and 27 women with a mean age of 56 (range 34–76) years) were treated. Twenty-one patients presented with recurrent disease. Forty-one patients already had been operated on before referral, with a mean of 2 (range 1–5) laparotomies. Seven patients had also received systemic chemotherapy previously, and one had been pretreated with intraperitoneal cisplatin. Two patients had previously undergone radiotherapy.

Surgery and heated intraperitoneal chemotherapy

In 33 patients the tumour deposits were spread over five or more abdominal regions, in ten the tumour was limited to two to four abdominal regions, and in three cases tumour was limited to only one region. Optimal surgical cytoreduction, leaving only tumour deposits smaller than 2·5 mm behind, was achieved in 40 patients. Major visceral resections were needed to achieve this, including partial gastrectomy, bowel resection, splenectomy, cholecystectomy, and ovariectomy and hysterectomy in women (*Table 1*). A mean of 2 (range 0–6) bowel anastomoses was needed per patient, while a colostomy was performed in 17 patients and an ileostomy in one. In 19 cases a (distal) gastrectomy with Billroth I reconstruction was performed, and in one patient partial gastrectomy was followed by a Billroth II reconstruction. The mean duration of operation was 10 (range 5·5–18) h, and the mean total estimated blood loss was 13 (range 1·6–55) litres.

In one patient a MMC dosage of 15 mg/m^2 was used, in two patients 30 mg/m^2, in 38 patients 35 mg/m^2 and in the remaining five patients 40 mg/m^2. In one patient the perfusion was stopped after 60 min because of severe diffuse bleeding. In all other patients the perfusion was performed as planned for 90 min. During and directly after the HIPEC procedure the MMC concentration in plasma and perfusion fluid was measured and the area under the curve (AUC) calculated. The mean ratio of perfusate : plasma AUC was 11·5. There was a wide interindividual variability in intraperitoneal MMC concentrations (3·6–25·8 g/ml), independent of the dosage used.

Postoperative course: morbidity and toxicity

The mean hospital stay was 31 (range 16–146) days. Major complications related to operation occurred in 18 patients, resulting in a morbidity rate of 39 per cent: stomach or bowel perforation (ten patients), enteral fistula (six), pancreatitis (one), pulmonary embolism (three), peripheral pressure neuropathy (five), pneumonia (three) and intra-abdominal or wound abscess (four). Eleven patients had to undergo reoperation for postoperative complications. Four patients died in the immediate postoperative period, resulting in an in-hospital mortality rate of 9 per cent. Causes of death were septic shock (three patients; in one case this occurred in combination with grade 4 leucocytopenia) and sudden death probably due to a pulmonary embolism (one patient). One patient developed multiple and persistent enteral fistulas for which he was treated by multiple bowel resections. This resulted in a short bowel syndrome, and the patient required prolonged parenteral feeding at home. All other patients resumed normal enteral feeding. MMC toxicity consisted mainly of bone marrow suppression. Twelve patients developed a grade 1 or 2 leucocytopenia (WHO Common Toxicity Criteria), and in ten patients grade 3 or 4 was observed. Thrombo-cytopenia

Table 1 Characteristics of resections needed to achieve optimal surgical cytoreduction

	Women (n=27)	Men (n=19)
Partial gastrectomy	8	12
Ileocaecal resection	16	12
Rectal resection	10	9
Hysterectomy	9	—
Splenectomy	12	12
Cholecystectomy	5	8

occurred in four patients. Well documented MMC toxicities such as nephrotoxicity and cardiotoxicity were not observed. Prolonged gastric paresis was seen in almost all patients, which delayed oral intake for a mean of 16 (range 5–33) days. This was probably due to the direct toxic effect of MMC and/or hyperthermia. No long-term toxicity related to MMC, such as haemolytic–uraemic-like syndrome, was observed.

Adjuvant chemotherapy and follow-up

Twenty-two patients received systemic chemotherapy with 5-FU–leucovorin after operation. In five of these patients this treatment was stopped because of intolerable toxicity ($n = 4$) or progressive disease ($n = 1$). After a median follow-up of 12 (range 1–43) months, 40 patients are still alive; eight have proven local recurrence. No distant metastases were observed. The mean interval between HIPEC and recurrence was 13 months. One patient died from recurrent disease 26 months after HIPEC; another died from an unrelated cause. The actuarial survival rate (Kaplan–Meier) at 2 and 3 years was 91 and 81 per cent respectively.

Discussion

During the past decade increasing attention was paid to HIPEC as a treatment option in patients with peritoneal carcinosis from different origins, such as colorectal carcinoma, gastric carcinoma, mesothelioma and sarcoma[13–16]. Theoretically, pseudomyxoma peritonei is always limited to the peritoneal cavity and should show no organ infiltration because of its benign character. Therefore, the surgical removal of tumour deposits should be possible and, because of its locoregional character, pseudomyxoma peritonei provides an ideal situation for additional locoregional (i.e. intraperitoneal) chemotherapy. The first clinical report on this treatment modality in pseudomyxoma peritonei was published in 1980 by Spratt *et al.*[8]. A significant survival benefit in patients treated by additional intraperitoneal chemotherapy compared with adjuvant systemic chemotherapy has been observed[11,17,18]. Since then, different treatment schedules have been used with regard to timing and duration of the perfusion, open or closed perfusion models, and the choice and dosage of chemotherapeutic agents. To optimize exposure of the surface of the abdominal contents to the perfusion fluid, peritoneal expansion is applied in some centres. This may be achieved by different methods[19]. In this report, a technique of peritoneal cavity expansion by elevation of the skin of the laparotomy wound using a retractor ring placed above the abdomen is described. The abdominal cavity is kept open, allowing the surgeon's hand to 'stir' the abdominal content during perfusion, resulting in better exposure of the peritoneal surfaces and more uniform distribution of drug and heat through the entire abdominal cavity. MMC was chosen as the chemotherapeutic agent because of its known activity in colorectal cancer, its direct cytotoxic effect, the thermal enhancement of its activity and penetration depth, and its favourable pharmacokinetics in HIPEC procedures[20,21].

Because the penetration depth of cytostatic drugs in tissue is limited, it is generally accepted that HIPEC should be combined with extensive cytoreductive surgery with the aim of leaving only limited macroscopic tumour deposits. Sugarbaker[12] introduced the technique of large peritonectomy procedures with the diathermy knife. The use of this technique combined with an aggressive surgical approach towards affected intra-abdominal organs made it possible to obtain optimal cytoreduction in most patients. However, this ultraradical cytoreductive approach in combination with HIPEC is associated with a significant surgical complication rate. In the series described here, four patients died after operation as a direct result of the treatment, resulting in a mortality rate of 9 per cent. This is comparable to the mortality rate after HIPEC reported by others (of up to 14 per cent) regardless of the technique and indication used[15,19,22–24]. However, the reported morbidity rate of 39 per cent appears to be relatively high compared with that observed by others after HIPEC procedures (up to 36 per cent[22,24]). One of the most important reasons for this is probably that, in this study, most patients had had extensive pretreatment. Postoperative surgical morbidity proved to be related mainly to the number of previous laparotomies and other intraperitoneal therapies, rather than to the intraperitoneal MMC concentration[25]. This finding has been confirmed by others[26]. Thus it seems likely that postoperative mortality and morbidity rates may be reduced substantially when patients are treated by cytoreductive surgery and HIPEC at primary diagnosis. Despite the high morbidity rate encountered, almost all patients had fully recovered by 6 months after operation. Haematological toxicity (bone marrow suppression) was observed in 48 per cent of patients. The latter is a result of the intraoperative chemotherapy and is related to the intraperitoneal MMC concentration. Although in most patients the leucopenia caused no extra morbidity, the combination of bone marrow suppression and surgical complications may be fatal. This was unfortunately demonstrated in one patient. No late toxicity related to MMC perfusion was observed. The significant morbidity associated with this aggressive regional treatment modality is probably one of the reasons for hesitating about the use of HIPEC and cytoreductive surgery in a comparatively benign disease such as pseudomyxoma peritonei. However, because of the poor long-term prognosis of pseudomyxoma treated with standard surgical therapy alone, with a 10-year survival rate of 10–30 per cent and considerable disease-related morbidity, the exploration of such an aggressive treatment modality in the search for better long-term results is justified[27].

An actuarial 3-year survival rate of 81 per cent after a median postoperative follow-up of approximately 12 months was observed, regardless of the histological character of the disease. Although the length of follow-up in this study was limited, the results are in accordance with those shown by Sugarbaker and colleagues, who found an overall 5-year survival rate of 60 per cent after HIPEC and radical surgery. When patients were divided into subgroups, these authors reported a 5-year survival rate of 86 per cent in patients with tumours with non-invasive histological features[10,11]. These results suggest that it might be important to look for more accurate (histological) prognostic factors[28].

To determine the precise benefit of HIPEC after radical surgery, randomized studies are needed. However, in a rare disease such as pseudomyxoma peritonei, this seems impossible to realize. The treatment of this condition should be centralized in order to reduce treatment-related morbidity and to improve the length of disease-free survival.

In conclusion, extensive cytoreductive surgery in combination with HIPEC and MMC is an aggressive locoregional therapy in patients with pseudomyxoma peritonei. Although this treatment modality is associated with considerable early postoperative mortality and morbidity rates, long-term results suggest that a better survival may be achieved than with conventional palliative surgery alone.

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Original article

Role of tumour necrosis factor in lung injury caused by intestinal ischaemia–reperfusion

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Background: Despite the well known inflammatory effects of tumour necrosis factor α (TNF), the mechanism of TNF-mediated lung injury following ischaemia–reperfusion (I/R) is still unclear. In this study, the role of TNF in the development of acute lung injury following intestinal I/R was investigated.

Methods: Male Wistar rats underwent either sham operation ($n = 10$), 1 h of superior mesenteric artery occlusion and 2 h of reperfusion (I/R, $n = 10$), or pretreatment with anti-TNF polyclonal antibody 2 mg/kg and I/R ($n = 6$). Lung injury was evaluated by Evans blue dye concentration, immunohistochemical staining and morphometric analysis. Intestinal injury was assessed by Evans blue dye concentration and histological examination.

Results: Intestinal I/R resulted in lung injury characterized by an increase in Evans blue dye concentration, neutrophil sequestration, and obvious staining for expression of pulmonary CD11b and CD18. Pretreatment of animals with anti-TNF antibody led to a reduction in the sequestration of neutrophils, and a decrease in expression of pulmonary intracellular adhesion molecule 1 and CD18. Anti-TNF antibody pretreatment also reduced the intestinal microvascular injury but not histological grade after intestinal I/R.

Conclusion: Treatment with an anti-TNF antibody resulted in a significant attenuation of lung injury following intestinal I/R. The data indicate that TNF is an important trigger for upregulation of pulmonary endothelial and neutrophil adhesion molecules after intestinal I/R.

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Introduction

Intestinal ischaemia–reperfusion (I/R) produces injury in both local and distant organs, including the lungs, kidneys and liver. Although several mechanisms have been proposed to explain the pathogenesis of the pulmonary injury after intestinal I/R, most attention has focused on the role of inflammatory leucocytes[1,2]. A growing body of evidence suggests a pivotal role for leucocyte–endothelial cell adhesion in the development of microvascular injury and organ dysfunction after I/R[3,4]. Adherence to endothelial cells causes neutrophils to release toxic substances that subsequently damage the endothelial layer, leading to an increase in capillary permeability and interstitial oedema.

The cytokine tumour necrosis factor α (TNF) has been reported to be an important modulator of pulmonary oedema in acute lung injury following I/R[5,6]. Experimental studies have suggested that plasma levels of TNF increase during intestinal I/R[7–13]. Other studies have demonstrated that blocking the biological effects of TNF by monoclonal or polyclonal antibodies reduces the severity of reperfusion-related injury[5,8]. Furthermore, acute lung injury with neutrophil sequestration and increased microvascular permeability is characteristic of experimental models in which intravenous TNF is administered[14].

Several studies have reported that TNF causes pulmonary injury after I/R. However, the role of TNF in generating and extending the lung injury following intestinal I/R is less clear. Sorkine *et al.*[5] noted that TNF blockade reduced the sequestration of neutrophils and microvascular leakage in the lungs of animals undergoing intestinal I/R. In contrast, Caty *et al.*[8] found that pretreatment with an anti-TNF antibody did not reduce the lung myeloperoxidase content, despite diminished microvascular leakage after intestinal I/R. Furthermore, Gaines *et al.*[13] reported that TNF did not contribute to pulmonary neutrophil infiltration in a rat hindlimb I/R model. In the present study, the role of TNF in the development of acute lung injury following intestinal I/R was investigated. The question of whether TNF-induced upregulation of adhesion molecules is important in the neutrophil-dependent phase of lung injury following intestinal I/R was also addressed.

Materials and methods

Experimental design

Animal care complied with the guidelines for the care and use of laboratory animals established by the Ethical Committee of University of Ankara, Turkey. Male Wistar rats weighing 220–320 g were fasted overnight and anaesthetized with an intraperitoneal injection of sodium pentobarbital 30 mg/kg. During the experiment animals were kept warm with a heating lamp. All animals were given Ringer's lactate solution 10 ml/kg. The superior mesenteric artery (SMA) was exposed through a midline abdominal incision and the rats were randomized into three groups. In group 1 (control, *n* = 10) the SMA was exposed but not occluded, and animals were followed for 1 h to simulate the ischaemic interval. The abdomen was then reopened and closed for 2 h to simulate the reperfusion interval in the other groups. In group 2 (I/R, *n* = 10) the SMA, as well as the collateral branches coming from the coeliac axis and the inferior mesenteric artery, were occluded with an atraumatic microvascular clamp (Vasculostat®; Scanlan International, St Paul, Minnesota, USA) for 1 h, followed by 2 h of reperfusion. In group 3 (anti-TNF, *n* = 6), animals were pretreated with polyclonal anti-TNF antibody 2 mg/kg (R & D Systems, Minneapolis, Minnesota, USA) 5 min before clamping (before ischaemia). These animals were then subjected to the same procedures as those in group 2. Intestinal I/R in this animal model was confirmed by the appearance of pulses in the marginal arteries using direct microscopic vision of the mesenteric circulation, as well as by intestinal fluorescein angiography in preliminary experiments. After injecting fluorescein 15 mg/kg through the penile vein, the mesenteric circulation was examined under a Wood's light in a darkened room.

Assessment of pulmonary microvascular injury

Intestinal I/R-induced pulmonary microvascular permeability was quantified *in vivo* by measuring the concentration of Evans blue dye in the lung tissue. Evans blue dye binds strongly to albumin and has been used as a marker of protein extravasation in models of acute lung injury[15]. At the time of initial laparotomy, immediately before SMA occlusion, Evans blue 30 mg/kg was injected into the penile vein. At the end of reperfusion, rats were heparinized (500 units) and a median sternotomy was performed. The ascending aorta was cut and the animals were killed. The hilus of the left lung was clamped and resected for immunohistochemical staining and histological examination. To eliminate residual blood and Evans blue dye from the pulmonary bed, the right lung was perfused via the pulmonary artery for 2 min with saline (37°C) at 0·04 ml per g body-weight per min using an infusion pump (Becton Dickinson, Brezins, France). Finally, the right lung was excised, rinsed externally with saline and blotted dry. The lung was weighed, placed in an oven and heated at 90°C for 16 h. The dried tissues were incubated in 2 ml of formamide at 37°C for 24 h. The dye concentration of the eluent was then measured by spectrophotometry at 620 nm. The concentration of Evans blue dye extracted from the lungs was expressed as micrograms of dye per milligram wet lung weight.

Assessment of pulmonary neutrophil sequestration

The upper half of each left lung specimen was removed and fixed in 10 per cent formalin solution for at least 24 h. Paraffin-embedded 4-µm sections were stained with haematoxylin and eosin and examined under a light microscope. A single pathologist, who was blinded to all groups, examined the pathological specimens. At least two different sections of each specimen were examined to determine the degree of injury. Lung polymorphonuclear neutrophil (PMN) sequestration was quantified by counting alveolar septal wall PMNs. Only peripheral lung parenchyma was examined. Microscopic fields containing other structures such as airways, large vessels and pleura were excluded. White blood cell entrapment was expressed as the mean number of PMNs per 10 high-power fields (HPF) (× 400).

Immunohistochemical analysis

Since intracellular cell adhesion molecule (ICAM) 1, CD11 and CD18 play an important role in neutrophil recruitment in the lung, immunohistochemical analysis was used to assess neutrophil–leucocyte interactions. Anti-CD11b and anti-CD18 staining was used to evaluate the neutrophil integrins in the pulmonary capillaries and ICAM-1 staining was used to evaluate their counterpart in the pulmonary endothelium.

The other halves of the lung specimens were placed in plastic boxes containing cryomatrix (10 per cent polyvinyl alcohol and 4 per cent polyethylene glycol) and wrapped with aluminium foil before snap freezing in liquid nitrogen. Frozen sections 5 µm thick were prepared and placed on poly L-lysine-coated slides. The slides were fixed in cold acetone for 10 min. The sections were kept at − 20°C until the staining procedure. A standard streptavidin–biotin complex method (Dako LSAB 2 peroxidase kit; Dako, Carpinteria, California, USA) with aminoethyl carbazole chromogen was used for immunohistochemical staining. Anti-CD11b and anti-CD18 staining was evaluated quantitatively. Positively stained PMNs were counted in five different fields using the × 40 objective (× 400 magnification with a Nikon Labophot™ microscope; Nikon, Tokyo, Japan) and the mean values were used in the statistical analysis. The staining intensity in endothelium was graded on five-point scale (0–4) for ICAM-1.

Assessment of intestinal injury

At the end of reperfusion after excision of the lung, two short segments of bowel were taken from the ileum. The samples were used for histological examination and Evans blue dye concentration measurement. The assessment of intestinal microvascular injury was done in the same manner as for lung tissue. Segments of small intestine for histopathological examination were fixed in 10 per cent formalin, and paraffin sections were stained with haematoxylin and eosin. Mucosal damage was graded using the criteria described previously by Chiu *et al.*[16].

Statistical analysis

Data are presented as median (range). Differences between the three groups were evaluated by Kruskal–Wallis one-way analysis of variance. When there was a significant difference among three groups, multiple comparison tests were used to define which groups differed from the others[17]. $P < 0.05$ was considered to be significant.

Results

Changes in pulmonary capillary permeability, as estimated by extravasation of Evans blue dye, were evaluated at the end of the experiment. Statistically significant differences were found among three groups ($\chi^2 = 7{\cdot}82$, 2 d.f., $P = 0{\cdot}02$). The concentration of Evans blue dye in the lungs of animals that underwent intestinal I/R was higher than that in controls (101·23 (144·72) *versus* 34·06 (49·23) µg/mg; $P = 0{\cdot}004$). There was no difference in lung dye concentration between animals pretreated with anti-TNF antibody and those that had intestinal I/R (64·53 (106·80) *versus* 101·23 (144·72) µg/mg; $P = 0{\cdot}28$). There was also no statistically significant difference between sham-operated animals and those treated with anti-TNF antibody, regarding Evans blue dye in the lung (34·06 (49·23) *versus* 64·53 (106·80) µg/mg; $P = 0{\cdot}08$).

Pulmonary neutrophil sequestration was investigated by measurement of pulmonary neutrophil morphometrics at the end of each experiment. Significant differences were found among three groups ($\chi^2 = 12{\cdot}58$, 2 d.f., $P = 0{\cdot}002$). There was a significant increase in PMN sequestration in the lungs after intestinal I/R compared with controls (3·00 (4·00) *versus* 0 (0) PMNs per 10 HPF; $P < 0{\cdot}001$). PMN sequestration in the lungs was lower in animals pretreated with anti-TNF antibody than in animals in the intestinal I/R group (1·50 (1·00) *versus* 3·00 (4·00) PMNs per 10 HPF; $P < 0{\cdot}05$).

Intestinal I/R resulted in the upregulation of leucocyte and pulmonary endothelial adhesion molecules (*Table 1*). There was a significant increase in ICAM-1 expression in the lungs harvested from animals undergoing intestinal I/R compared with controls ($P = 0{\cdot}03$). ICAM-1 expression was lower in animals pretreated with anti-TNF antibody than after intestinal I/R alone ($P = 0{\cdot}002$). There was no statistically significant difference in ICAM-1 expression between rats pretreated with anti-TNF and controls. While there was minimal staining for leucocyte CD11b in the lungs of sham-operated animals, there was obvious staining in animals that had sustained intestinal I/R ($P = 0{\cdot}002$). Pretreatment with anti-TNF antibodies before intestinal I/R did not diminish the expression of pulmonary leucocyte CD11b ($P = 0{\cdot}11$). Leucocyte CD18 was upregulated in the lungs of animals subjected to intestinal I/R ($P < 0{\cdot}001$). Pretreatment with anti-TNF antibody before intestinal I/R resulted in decreased expression of CD18 compared with that seen after intestinal I/R alone ($P = 0{\cdot}004$) (*Table 1*).

Changes in intestinal capillary permeability were estimated by measurement of extravasation of Evans blue dye. Statistical analysis revealed a significant difference among the three groups ($\chi^2 = 11{\cdot}22$, 2 d.f., $P = 0{\cdot}004$). After 2 h of reperfusion, animals subjected to intestinal I/R had significant increase in Evans blue dye concentration in the intestine compared with sham-operated animals (716·22 (868·25) *versus* 30·34 (92·02) µg/mg; $P < 0{\cdot}001$). Pretreatment with anti-TNF antibody protected the animals from reperfusion-induced capillary leak (129·08 (61·30) *versus* 716·22 (868·25) µg/mg in animals subjected to intestinal I/R; $P = 0{\cdot}06$). There was no statistically significant difference between sham-operated animals and those pretreated with anti-TNF antibody (30·34 (92·02) *versus* 129·08 (61·30) µg/mg; $P = 0{\cdot}06$).

Intestinal mucosal injury was evaluated by histological examination of the intestine. Significant differences were found among three groups ($\chi^2 = 13{\cdot}72$, 2 d.f., $P = 0{\cdot}001$). Intestinal I/R resulted in mucosal lesions with a histological grade of 4·00 (3·00) compared with 0 (0) in controls ($P < 0{\cdot}001$). Pretreatment with anti-TNF antibody did not reduce the severity of intestinal injury (4·50 (1·00))

Table 1 Expression of adhesion molecules in leucocytes and pulmonary endothelial surface

	Control (n=7)	Intestinal I/R (n=10)	Anti-TNF pretreatment (n=6)	ANOVA χ^2	d.f.	P
ICAM-1	2·00 (3·00)*	3·00 (1·00)	2·00 (2·00)†	9·00	2	0·011
CD18	19·00 (18·00)§	54·00 (25·00)	33·00 (23·00)‡¶	12·82	2	0·002
CD11b	24·00 (29·00)†	42·00 (35·00)	35·50 (15·00)	8·58	2	0·014

Values are median (range). Immunohistochemical analysis was performed on seven animals in the control group. ICAM, intracellular adhesion molecule; I/R, ischaemia–reperfusion; TNF, tumour necrosis factor α; ANOVA, Kruskal–Wallis analysis of variance. $^*P = 0·03$, $^†P = 0·002$, $^‡P = 0·004$, § $P < 0·001$ *versus* intestinal I/R; ¶$P = 0·004$ *versus* control

compared with that seen after intestinal I/R alone ($P > 0·05$). This degree of mucosal injury was significantly greater than that in sham-operated animals ($P < 0·001$).

Discussion

In this study, reperfusion after 1 h of intestinal ischaemia induced lung and intestinal injury characterized by increased permeability and neutrophil sequestration. Previous studies have established the ability of neutrophils to mediate remote organ injury after intestinal I/R by rendering animals neutrophic or by blocking neutrophil adhesion to vascular endothelium. The latter attenuates organ injury[2,4]. The present data reinforce the observation that activated neutrophils contribute to lung injury. Thus, blocking TNF activity by administration of polyclonal antibodies to TNF decreased both neutrophil adhesion to pulmonary capillaries and lung neutrophil sequestration. These findings corroborate those of Welborn *et al.*[7], who reported that abolishing TNF activity with a TNF-binding protein and anti-TNF antibody decreased lung neutrophil recruitment and capillary membrane changes in mice following intestinal I/R. Although TNF concentrations were not measured here, it is known that levels of TNF in the systemic and portal circulation increase during intestinal I/R[9,18]. Furthermore, Wyble *et al.*[9] demonstrated that TNF is present in the venous effluent of reperfused intestine in concentrations capable of increasing expression of endothelial cell adhesion molecules. TNF blockade has been shown to prevent shock, low cardiac index and vascular hyporeactivity after I/R[11,12].

The blockade of TNF reduced the expression of CD18 in leucocytes and ICAM-1 in pulmonary endothelium. Indeed, Seekamp *et al.*[3] reported that anti-TNF antibodies attenuated E-selectin expression in the lung vasculature in a rat hindlimb I/R model. Similarly, Colletti *et al.*[19] found that TNF blockade reduced pulmonary ICAM-1 expression after hepatic I/R, while Squadrito *et al.*[11] reported that TNF blockade reduced ICAM-1 expression in the aorta and SMA. To arrest the rolling phase and mediate firm adhesion, neutrophil integrins including CD11a/CD18 and CD11b/CD18 interact with members of the immunoglobulin gene superfamily, especially ICAM-1. This moiety also has a central role in neutrophil transmigration into the interstitium. In other investigations of intestinal I/R, blocking adhesion molecule activity has also afforded substantial protective effects. Blocking antibodies against ICAM-1, CD11 and CD18 decrease tissue injury in the setting of reperfusion in the liver[19], heart[20] and skeletal muscle[21]. In this study the evidence suggests that the upregulation of adhesion molecules was mediated by TNF. The data support the concept that ICAM-1, CD11 and CD18 play an important role in neutrophil recruitment in the lung and that these neutrophils may contribute to the development of lung injury following intestinal I/R. Research shows that TNF enhances expression of ICAM-1, E-selectin and vascular cell adhesion molecule 1 in endothelial cells and upregulates leucocyte CD18 complex[3,10,22,23]. Wyble *et al.*[9] demonstrated that TNF and IL-1 released from human intestine during I/R cause ICAM-1 and E-selectin upregulation in cultured human endothelial cells.

Intestinal I/R is associated with a generalized inflammatory response culminating in acute lung oedema. In the present study, blockade of TNF reduced lung neutrophil sequestration and expression of leucocyte–endothelial adhesion molecules during intestinal I/R. However, the increase in lung microvascular permeability, measured by Evans blue dye concentration, was not prevented completely by anti-TNF antibody. The data indicate that the microvascular changes in the lungs during intestinal I/R are not completely related to neutrophils or TNF. Other factors, including endothelial dysfunction, diminished basal nitric oxide release from pulmonary vascular endothelium, pulmonary arterial vasoconstriction and other mediators, may also be important in the development of lung oedema after intestinal I/R[2,24]. On the other hand, pretreatment with an anti-TNF antibody prevented the intestinal microvascular injury, although mucosal injury was not abolished. These findings may also suggest that the acute intestinal injury after I/R is largely TNF mediated, in contrast to the lung microvascular injury which occurs independently of TNF. Although this does not exclude the possibility of a dose-related event, it is possible that other, undefined mediators are also operative in this setting.

Acknowledgements

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Correspondence

The Editors welcome topical correspondence from readers relating to articles published in the Journal. Letters should be no more than 250 words in length and should be typed on A4-sized paper in double spacing

Causes of re-recurrence after polytetrafluoroethylene patch saphenoplasty for recurrent varicose veins (*Br J Surg* 2000; 87: 1356–60)

Sir

One of the aims of the study was 'to determine whether polytetrafluoroethylene (PTFE) patch saphenoplasty was effective in preventing recurrent veins at the level of the patch' but this was not realised. The authors state that 'deep venous disease was identified in 16 legs ... All but two patients had no change in the deep veins following surgery'. It is not clear why patients were operated upon in spite of the presence of deep venous disease in the preoperative duplex imaging. The cause of the development of non-occlusive deep vein thrombosis after surgery in one of the patients is unexplained.

'Recurrence was more likely in patients with deep venous incompetence (six of 16) than in those with normal deep veins (10 of 54)'. This statement is statistically incorrect. 'Recurrent varicose veins caused by failure of the operation at the level of the patch were uncommon' seems to contradict with the later statement that 'failure at groin level is still the most significant cause of recurrent veins'.

The fact that 'none of the patients wanted further treatment at the time of review' does not mean that they were happy with the outcome.

The basis on which this procedure was judged to be safe in the present study is not clear. This is despite the fact that three patients developed wound infection and two developed signs of deep venous disease after the procedure.

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Authors' reply

Sir

Firstly, before a randomized trial could be undertaken the safety of the patch needed to be established. Wound infections were no more frequent in the present report than in our previous study[1]. The issue of deep vein thrombosis (DVT) is important. Fourteen of the 16 patients with deep venous disease before surgery had localised deep venous incompetence (ten femoral, four popliteal). All were counselled about the risk of perioperative DVT and received thromboprophylaxis. None had clinical evidence of DVT. The two patients with late changes were asymptomatic; one had popliteal reflux and the other common femoral vein reflux. Although subclinical perioperative DVT cannot be excluded the follow-up scans were performed 30 and 38 months postoperatively and the changes could have occurred at any stage during that interval.

This paper does not provide definitive answers about the aetiology of re-recurrent long saphenous veins. Failure can occur at the level of the patch and at groin level where upper thigh tributaries or perforating veins dilate after saphenofemoral disconnection. In the present study the number of upper thigh perforating veins seen on varicography was surprising and note that the PTFE patch could have suppressed the development of neovascularization.

We did not use sophisticated measures of patient satisfaction or quality of life, but 88 per cent of patients remained satisfied with the results of their surgery. By keeping patients under review we actually increased the number of reoperations and previously 20 per cent of patients in the control arm requested reoperation[1]. No patient with a patch in five years of study in Gloucester has yet required groin re-exploration for recurrent veins.

We believe that the results reported in our paper justify the randomized trial of patch saphenoplasty now being undertaken.

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1 Dwerryhouse S, Davies B, Harradine K, Earnshaw JJ Stripping the long saphenous vein reduces the rate of reoperation for recurrent varicose veins. five-year results of a randomized trial. *J Vasc Surg* 1999; 29: 589–92.

Cost-effective carotid endarterectomy (*Br J Surg* 2000; 87: 323–7)

Sir

This study demonstrated reduced hospital stay, intensive care admission and perioperative investigations without increasing the incidence of complications. However, general anaesthesia and transcranial Doppler monitoring were used in all patients with no mention of the number of carotid shunts. Local anaesthesia and transcranial Doppler equipment is more expensive and less accurate than neurological monitoring of the awake patient[1,2]. Monitoring the awake patient decreases the need for shunting[3]. Use of a shunt is costly and it generally prolongs the operating time. General anaesthesia may cause more perioperative haemodynamic fluctuations requiring careful monitoring and treatment which may increase cost; this occurred in five patients[4]. In a third-world Caribbean setting, with limited facilities, restricted budgets and minimal monitoring equipment, we perform carotid endarterectomy safely under local anaesthesia and have had to shunt only one patient in 82 consecutive cases, we encountered one permanent neurologic complication and most patients were discharged within 24 hours[5]. Three patients were converted to general anaesthesia, two because of discomfort and one because of intravascular injection of local anaesthesia. Even where excellent facilities exist, workers like Sandison *et al.* may further improve their cost-effectiveness by using local anaesthesia.

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5 Naraynsingh V, Kuruvilla T, Maharaj D, Rampaul R. Carotid endarterectomy under local anaesthesia: the technique of choice for developing countries *West Indian Med J* 1998; 47(suppl.): 13.

Author's reply

Sir

I thank Naraynsingh *et al.* for their interest in our paper. We have also started to use local anaesthesia to perform carotid endarterectomy during the last 2 years and have contributed to the GALA trial, a multicentre UK study comparing general with local anaesthesia. However, in our experience the dissection phase takes longer with local anaesthesia than with general. Even using regional blocks supplemented with superficial infiltration, some areas require further infiltration, particularly those adjacent to the carotid artery. The authors state that the use of a shunt prolongs the operation, and, in our experience, the same is true of local compared with general anaesthesia. We agree that the use of local anaesthesia reduces the requirement for a shunt. In our experience 160 out of 244 patients (40 per cent) required a shunt under general anaesthetic compared with 2 out of 31 (7 per cent) for local.

We disagree with their dismissal of transcranial Doppler monitoring, which we find useful for two reasons. First, it can be used to detect a reduction in flow in the middle cerebral artery and, second, it can detect emboli. The first use can be important to indicate which patients require a shunt during general anaesthesia, but is not important if local anaesthesia is used apart from the rare occasions when conversion to general anaesthetic is required. In common with their experience, we have had to convert four patients from local to general anaesthesia, usually because of discomfort caused by retraction to gain access to the higher reaches of the internal carotid artery. We have also found that, in patients with a high degree of internal carotid stenosis, the opening of self-retaining retractors can completely occlude the flow in the middle cerebral artery, which can be restored by reducing the degree of retraction by a small amount. The second use, the detection of emboli, remains important despite the type of anaesthesia. Particulate emboli are most likely to cause neurological sequelae, and may be precipitated during the dissection of the carotid bifurcation. Detection of emboli during the prearteriotomy phase alerts the surgeon to take extra care to dissect the body off the arteries. Sometimes just pressing on the skin with a swab is sufficient to send a shower of emboli into the cerebral circulation from an unstable plaque. Such patients require great care to minimize manipulation of the carotid and therefore the embolic load. If such emboli cause a neurological deficit during local anaesthesia the effect may be observed immediately, but the damage has been done rather than avoided. We believe the combination of local anaesthesia with transcranial Doppler monitoring of the middle cerebral artery offers the best form of protection during carotid endarterectomy.

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Mesh compared with non-mesh methods of open groin hernia repair: systematic review of randomized controlled trials (*Br J Surg* 2000; 87: 854–9) *and* Laparoscopic compared with open methods of groin hernia repair: systematic review of randomized controlled trials (*Br J Surg* 2000; 87: 860–7)

Sir

The EU Hernia Trialists Collaboration has answered a number of outstanding questions related to hernia surgery and should be congratulated. The first paper demonstrates that, in terms of hernia recurrence, the open mesh repair with a recurrence rate of 1·4 per cent is the 'gold standard' against which all other techniques should be compared. The second paper proves that time to return to normal activities, recovery time and analgesia usage are all reduced by the laparoscopic approach. Unfortunately, the paper also concludes that there is a small, but significant, excess of serious complications after laparoscopic repair.

This apparent failing warrants further examination with respect to the totally extraperitoneal (TEP) approach. The TEP repair is safe with no serious complications as reported in this paper. All serious complications occurred in patients undergoing a transabdominal preperitoneal (TAPP) laparoscopic repair. If the two types of laparoscopic repair had been analysed separately the complication rate for TAPP would have been much higher than the reported rate of 4·7 complications per 1000 patients (approximately 7·5 per 1000). Currently the TEP approach is unfairly judged because of failure to distinguish between the two types of laparoscopic repair. Future studies should compare the open mesh repair with the two types of laparoscopic approach separately.

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Letter 2

Sir

I read the article on laparoscopic compared with open methods of groin hernia repair with interest. While congratulating the authors for this study in general, I fail to see how they can draw worthwhile conclusions from a series that has been followed up for only 6 days (the study by Schmitz *et al.* from Germany, as quoted in *Table 1*). The authors were obviously interested in the recurrence rate as the best measure of outcome but surely a follow-up of 6 days is woefully inadequate.

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Letter 3

Sir

We read with interest the review of the published randomized trials of laparoscopic compared with open methods of groin hernia repair and wish to raise the following points.

Firstly, we notice a trend in the trials that report an early return to work after laparoscopic procedures (range 13–15 days) also show an earlier return to work with the open procedure (range 17–21 days). Conversely, those that show a late return to work after laparoscopic repair (range 21–29 days) demonstrate a congruent delayed return to work after open surgery (range 28–44 days). One might speculate that this is due to cultural reasons or biases within the study design. The trials from Scandinavian countries appear to show an early return to work with either method.

Secondly, there are six trials cited in which return to work is reported as approximately twice as long following open surgery (range 21–35 days) compared with laparoscopic surgery (range 10–17 days), though it is important to note the open procedure in all these trials employed a non-mesh technique. Indeed, in their review of open mesh versus non-mesh repair, the collaborators report that return to work is earlier in the mesh group where a tension-free repair has been used.

It would be reasonable to conclude that the main differences in recovery are due to divergent social and cultural expectations and regional variations in the health delivery system rather than the actual mode of repair.

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Authors' reply

Sir

We are grateful to the respondents for their interest in our papers. Although we did report in the discussion section that all potentially serious complications occurred in patients undergoing the transabdominal preperitoneal (TAPP) laparoscopic repair, there were some reasons for us not to stress this finding. We reviewed randomized controlled trials that compared laparoscopic with non-laparoscopic methods of repair. The number of trials that tested the totally extraperitoneal (TEP) laparoscopic method is relatively small in comparison with the number that tested the TAPP approach. Furthermore, surgeons who performed the TEP method in the trials had often first gained laparoscopic experience with the TAPP repair. We prefer to draw more solid conclusions when there are more data available. Members of the EU Hernia Trialists Collaboration are currently contributing individual patient data from their trials and analysis of these will enhance the evidence presented in our paper, including that relating to serious complications.

Of course we agree with Dr Wijesuriya that the follow-up period reported for the trial by Schmitz *et al.* does not contribute to the assessment of subsequent recurrence risk. This is why the trial does not appear in *Figure 1* of this paper.

All our comparisons have been made within randomized controlled trials (and not between trials). While we agree that differences between trials reflect local circumstances, such as clinical advice and cultural expectations, these will not explain the within trial differences. The striking finding was the consistency of within trial differences, such as return to usual activities; this suggests that the actual mode of repair also affects recovery.

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Functional outcome after restorative panproctocolectomy for ulcerative colitis decreases an otherwise enhance quality of life (*Br J Surg* 2000; 87: 802-7)

Sir

This study concluded, from selected disease-specific and general quality of life measures, that the functional outcome of ileal pouch-anal canal anastomosis appeared significantly worse than Koch's or Brooke's pouch although body image was better with the former.

The use of rank order to compare altered bowel emptying with other quality of life measures such as noise, clothing or sexual relations are neither insightful nor reflective of the actual improvement of function after surgery. The seven quality of life measures represent different domains and should not be ranked alongside each other. A more useful measure would be to compare patients' score before and after operation. This method would have provided an improved score after operation, reflecting an improved quality of life. This would be most marked in patients undergoing ileal pouch-anal canal anastomosis as they would have none of the original disease symptomatology while retaining normal anal faecal expulsion without the need for an abdominal stoma.

The discussion failed to address the fact that the study may have involved a heterogeneous group of patients. Detailed patient profiles are required, especially in the ileal pouch subgroup, where the presence of patients with pouchitis with increased frequency of stools would decrease the functional score of altered bowel emptying.

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Authors' reply

Sir

There are valid concerns that current quality of life measures may not demonstrate obvious differences. We are not the first to fail to show a quality of life advantage for pouch surgery over an end-ileostomy[1-3]. Either these studies are correct, in which case the reasons need to be explored and addressed (as we have done in our paper) or there are differences and the methodology has been insensitive or inappropriate. This may be for practical reasons some of which are mentioned by Gee and Seow-Choen. It may be because what patients want and what quality of life actually measures are not the same.

Function and quality of life are quite different. Quality of life contains many different domains usually ranked despite differences, as in the SF-36. We accept Gee and Seow-Choens' desire to present a paper which would prospectively document quality of life before and after surgery over a sufficiently long period to generate the numbers necessary to make meaningful comparisons. We have, however, confirmed the work of others.

We consider the points made on pouchitis as spurious: pouchitis is an integral consequence of the decision to have a pouch in some patients which does not arise in patients with an ileostomy. This might legitimately affect quality of life adversely as a consequence of the decision to have a pouch.

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1 Jimmo B, Hyman NH. Is ileal pouch-anal anastomosis really the procedure of choice for patients with ulcerative colitis? *Dis Colon Rectum* 1998; **41**: 41–5.
2 Weinryb RM, Gustavsson JP, Liljeqvist L, Poppen B, Rossel RJ. A prospective study of the quality of life after pelvic pouch operation. *J Am Coll Surg* 1995; **180**: 589–95.
3 McLeod RS, Churchill DN, Lock AM, Vanderburgh S, Cohen Z. Quality of life of patients with ulcerative colitis preoperatively and postoperatively. *Gastroenterology* 1991; **101**: 1307–13.

Erratum

M. S. Rodgers and J. L. McCall. Surgery for colorectal liver metastases with hepatic lymph node involvement: a systematic review. *Br J Surg* 2000; **87**: 1142-55. Please note that in *Table 1* the overall survival rate for the paper by Nordlinger *et al.*[139] is 28% 5-year actuarial, not 8% as stated. The Journal apologizes for this error.

Thyroid Cancer: Diagnosis and Treatment

O.H. Clark, S. Noguchi (eds)

260 × 185 mm. Pp. 447 Illustrated. 2000 St Louis: Quality Medical Publishing. $99 00

The editors of this book are internationally recognized endocrine surgeons on the grounds of their surgical expertise and research activity in thyroid cancer. For this book they have collected together authors from the USA, Europe and Asia to produce a work divided into five sections. These include: (1) Epidemiology, Cytology and Histology of Thyroid Cancer; (2) Imaging Tools and Technology; (3) Decision Making: selecting a surgical approach; (4) Management; and (5) Advances in Molecular Biology. The text is well illustrated with photographs and, where appropriate, graphs and tables. Each chapter is well researched and ends with a conclusion that is brief and to the point. Controversies in the management of thyroid cancer are clearly discussed and opposing views given appropriate prominence. This book is an up-to-date reference source and is suitable for browsing to get the feel of issues that lie within the remit of other specialist disciplines. It will be of interest to all those involved in the care of patients with thyroid cancer, a core text for any multidisciplinary team. This is a highly satisfying book to read and gave great enjoyment to this reviewer.

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CD-ROM Pantogens: Prime Skills in Surgery; Current Techniques in Surgery – Laparoscopic Cholecystectomy; Current Techniques in Surgery – Open Repair of Inguinal Hernia (Adult)

M. H. Edwards

Darlington Scalpel Information Systems £59·95 each

A pantogen is a computer programme that seeks to provide 'all the gen' with regard to training in operative surgery. There are three programmes reviewed here, one called *Prime Skills in Surgery* and two programmes of *Current Techniques in Surgery* covering laparoscopic cholecystectomy and open repair of adult inguinal hernia. The programmes are provided on CD-ROM and require a PC with at least a 486 processor, quad speed compact disc player, sound board and speakers, Windows 95, Word for Windows and printer.

The *Prime Skills in Surgery* programme is interactive and requires the trainee to have available basic surgical equipment such as gloves, scalpel, scissors, suture material, haemostats and a padded jiffy bag for practising suturing techniques. There are timed exercises as well as instructions on suturing incisions in the jiffy bag, which I found somewhat unrealistic as a simulation and yet easily available. The format is highly didactic, which is suitable for junior surgical trainees, and includes video clips, still photographs and an accompanying voice over. The quality of the voice over varies and it is a pity that a professional was not employed for this role. My major criticism relates to actual techniques as depicted on the video clips. The suturing techniques are clear and satisfactory but the knotting techniques are not. The one-handed knot demonstrated is not a true reef knot as the hands do not cross and the index finger knot is repeated rather than alternating index finger and middle finger knots. Teaching such practice is dangerous and is not good technique. The same applies for the instrument knot in which the throws are not laid correctly as demonstrated. Furthermore, there are photographs of the needle being held in the fingers, which in this day of virally transmitted disease, is not a safe practice or one that should be encouraged. These may appear to be minor aspects of technique, but in the training of junior surgeons they are crucial as it is important for operators to pick up good practice early and not have to unlearn bad practice later.

The programmes on *Current Techniques in Surgery* are different in that they demonstrate a logical step-by-step approach to two common surgical procedures, namely laparoscopic cholecystectomy and open inguinal hernia repair. Inevitably there will be minor individual variations in technique between surgeons who regularly perform these procedures, but the chosen approach is most applicable to junior trainees, who are taken through these procedures step-by-step in a clear and didactic manner. The programmes also cover issues such as complications and anatomical variations and are well illustrated. I was surprised that the principles and practice of operative cholangiography were excluded from the laparoscopic cholecystectomy programme. However, there were useful inclusions in both programmes of 'Information to Patients' about their operation and what to expect from admission to discharge. These could usefully be adapted for any institution and any individual, with local requirements included.

Overall these programmes will be of significant interest to all junior surgical trainees. They will help a trainee concentrate on acquiring the necessary technical skills and, if combined with work on simulations, will help them develop manual dexterity. However, attention to detail is important and my disappointment relates to such basic skills as knot tying and handling of sharps which are unacceptable. In spite of these reservations I am certain that such an approach to surgical training will prove valuable. If the above issues were to be corrected in the next version of these programmes I would be happy to recommend highly these teaching tools to surgical trainees.

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Scientific Surgery

The *British Journal of Surgery* is committed to practice of surgery based on scientific evidence. Each month we will publish a list of randomized trials and meta-analyses collated from English-language publications. A collection of all these papers will be available in the Scientific Surgery Archive which will be held on the *British Journal of Surgery* website together with links to free Medline sites where the full papers can be accessed (see below for details). If you have published a recent randomized trial which has not been featured in Scientific Surgery, the Editors would be pleased to receive a reprint and consider its inclusion.

Stacey MC, Mata SD, Trengove NJ, Mather CA. Randomised double-blind placebo controlled trial of topical autologous platelet lysate in venous ulcer healing. *Eur J Vasc Endovasc Surg* 2000; **20**: 296–301

There was no improvement in the rate of healing using weekly application of platelet lysate in this trial including 86 patients with a venous ulcer

Wright DM, Hall MG, Paterson CR, O'Dwyer PJ. A randomized comparison of driver reaction time after open and endoscopic tension-free inguinal hernia repair. *Surg Endosc* 1999; **13**: 332–4.

Recovery of driver reaction times was slower after open repair (slower reaction times on postoperative days one and three), however, all 64 patients in the study were back to normal after 1 week

Harling R, Moorjani N, Perry C, MacGowan AP, Thompson MH. A prospective, randomised trial of prophylactic antibiotics *versus* bag extraction in the prophylaxis of wound infection in laparoscopic cholecystectomy. *Ann R Coll Surg Engl* 2000; **82**: 408–10.

There were three wound infections in each group in this study including 76 operations.

Messori A, Trippoli S, Vaiani M, Gorini M, Corrado A. Bleeding and pneumonia in intensive care patients given ranitidine and sucralfate for prevention of stress ulcer: meta-analysis of randomised controlled trials. *BMJ* 2000; **321**: 1103–6.

Eighteen studies were included in several categories but numbers were still too small for definitive answers. Ranitidine appears ineffective and may increase the rate of pneumonia, studies on sucralfate were inconclusive.

The oral Iloprost in Severe Leg Ischaemia Study Group. Two randomised and placebo-controlled studies of an oral prostacyclin analogue (Iloprost) in severe leg ischaemia. *Eur J Vasc Endovasc Surg* 2000; **20**. 358–62.

In two studies containing a total of 802 patients with severe leg ischaemia, there was no improvement in limb salvage or death rates from low or high dose oral Iloprost. There was an improvement in the composite endpoint of survival without ischaemic necrosis and without the need for regular analgesia.

Zelenitsky SA, Silverman RE, Duckworth H, Harding GKM. A prospective, randomized, double-blind study of single high dose *versus* multiple standard dose gentamicin, both in combination with metronidazole for colorectal surgical prophylaxis. *J Hosp Infect* 2000; **46**: 135–40.

Some 146 patients were included; deep infection rates were similar for single high (6/74, 8 per cent) and standard multiple dose gentamicin (5/72, 7 per cent).

Bonithon-Kopp C, Kronborg O, Giacosa A, Räth U, Faivre J for the European Cancer Prevention Organization Study Group. Calcium and fibre supplementation in prevention of colorectal adenoma recurrence: a randomised intervention trial. *Lancet* 2000; **356**: 1300–6.

Some 665 patients were divided into three groups and followed for 3 years. On colonoscopy, recurrent adenomas were found in 20 per cent of control subjects, compared to 16 per cent who had additional calcium and 29 per cent in those who had additional dietary fibre.

Chapman WC, Clavien P-A, Fung J, Khanna A, Bonham A. Effective control of hepatic bleeding with a novel collagen-based composite combined with autologous plasma. Results of a randomized controlled trial. *Arch Surg* 2000, **135**: 1200–4.

Some 67 patients undergoing partial hepatectomy had bleeding controlled with a collagen sponge; 38 had additional composite sprayed on the surgical site The composite significantly reduced the median time to haemostasis from 360 to 150 s, $P < 0\ 001$

Derry S, Loke YK. Risk of gastrointestinal haemorrhage with long-term use of aspirin· meta-analysis. *BMJ* 2000; **321**: 1183–7.

This meta-analysis included 24 randomized trials There was no evidence that the risk of bleeding was dose-relatec, or that it was reduced by the use of modified release formulations

The *British Journal of Surgery* website can be found at *www.bjs.co.uk*. In addition to details of current and previous articles featured in Scientific Surgery, the website contains details of the Editorial Team and Board, instructions to prospective authors, links to other sites (such as The Cochrane Library) and forthcoming surgical events. The Editors welcome comments and suggestions about the website, either in writing or through the website address.

Association of Upper Gastrointestinal Surgeons of Great Britain and Ireland

The following abstracts were presented to the 4th Annual Scientific Meeting of the Association of Upper Gastrointestinal Surgeons of Great Britain and Ireland on 21–22 September 2000. The *British* Journal of Surgery prize was awarded to the abstract entitled 'High risk of surgical intervention in hereditary pancreatitis' by N. Howes, M. M. Lerch, J. Mössner, S. Endres, J. Deviere, G. Verreman *et al.*

European Study Group for Pancreatic Cancer-1 interim results: a European randomized study to assess the roles of adjuvant chemotherapy and chemoradiation in resectable pancreatic cancer

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Background: Pancreatic cancer affects 8–12 per 100 000 population per year in Europe. Following resection, the long-term survival rate is only 10–15 per cent and the role of adjuvant treatment is uncertain. The aims of the study were to answer two questions: (1) whether there is a role for chemoradiation (40 Gy and 5-fluorouracil (5-FU), and (2) whether there is a role for chemotherapy (5-FU–folinic acid (FA)) weekly).

Methods: A multicentre European prospective randomized controlled trial was organized by the European Study Group for Pancreatic Cancer (ESPAC). A 2 × 2 factorial design was used, asking both questions of the same patient, and a pragmatic design asking only one of the two questions of each patient. The data were reviewed at regular intervals by the Independent Data and Safety Monitoring Committee (IDSMC).

Results: Some 531 patients with pancreatic ductal adenocarcinoma were randomized from 80 clinicians in 11 countries. Randomization was stratified by resection margin involvement; 82 per cent of patients were negative. Some 239 patients (45 per cent) are alive to date, at a median follow-up of 9 (interquartile range 1–24) months. Preliminary results show no evidence of a benefit for chemoradiation treatment (median survival 14 months with chemoradiation *versus* 15·7 months without; $P = 0·24$). There is some evidence of a survival benefit for patients having chemotherapy (median survival 19·5 months *versus* 13·5 months with no chemotherapy; $P = 0·003$). The effect is reduced when taking into account whether patients received radiotherapy ($P = 0·01$), indicating that radiotherapy may reduce the overall benefit of the chemotherapy. The IDSMC recommended closing recruitment to the chemoradiotherapy arm.

Conclusion: There is no role for adjuvant chemoradiotherapy in pancreatic cancer, but there may be a role for chemotherapy. ESPAC-3 is now randomizing between (1) surgery alone, (2) 5FU–FA and (3) gemcitabine.

High risk of surgical intervention in hereditary pancreatitis

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Background: The aim of the study was to quantify the risk of surgical intervention in families with hereditary pancreatitis referred to the European Registry of Hereditary Pancreatitis and Familial Pancreatic Cancer (EUROPAC).

Methods: Recruitment to EUROPAC started in 1997; families were considered if two or more members had chronic pancreatitis of unknown aetiology. Families were tested for protease serine 1 (*PRSS1*) mutations using polymerase chain reaction restriction digestion, sequencing the *PRSS1* gene-screened negative families. Clinical information about surgical intervention was obtained from family members and referring clinicians, and the cumulative incidence of surgical intervention in the N29I and R122H mutation groups was determined with the Kaplan–Meier method.

Results: Forty-four families had the R122H mutation, 21 the N29I mutation, and 31 were negative for both. Some 14 (35 per cent) of 40 patients (median age 26·5 (95 per cent confidence interval (c.i.) 21·5–36·3) years) with the N29I mutation had an operative procedure for complications of pancreatitis, compared with 21 (25 per cent) of 83 patients (median age 24 (95 per cent c.i. 13·8–35·8) years) with the R122H variant. This resulted in a cumulative lifetime risk for surgical intervention of 0·6 (95 per cent c.i. 0·5–0·8) for the R122H mutation and 0·6 (0·5–0·8) for the N291 mutation ($P = 0 \cdot 11$, log rank test).

Conclusion: Hereditary pancreatitis carries a significantly increased risk of surgical intervention in this cohort of patients, which equates to an estimated lifetime risk of around 60 per cent. The risk of surgery appears to be independent of *PRSS1* mutation type.

Initial results with a minimally invasive technique of pancreatic necrosectomy

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Background: The aim of the study was to evaluate the initial results with a new technique of minimally invasive pancreatic necrosectomy (MIPN).

Methods: A retrospective audit was carried out of pancreatic necrosectomies performed on one unit from October 1996. Patients were divided into two groups: those admitted before November 1998 who underwent a conventional open necrosectomy and those admitted after this date who were considered for treatment with MIPN.

Results: Thirty-one patients underwent pancreatic necrosectomy, of which 20 cases were tertiary referrals. Thirteen patients (median age 51 (range 33–77) years; ten men, three women) admitted before November 1998 underwent a conventional open technique; seven of these patients died. Since then, 18 patients (median age 59 (range 33–74) years; ten women, eight men) have undergone necrosectomy, 12 by MIPN (median of 3 (range 1–6) procedures) and six by an open technique. Reasons for using an open technique included a left renal adenocarcinoma (one patient), poor access route (three patients) and failure to insert a guidewire under computed tomographic control (two patients). There were two deaths during this later period ($P < 0 \cdot 05$, Fisher's exact test in comparison with the earlier time period). In patients who were discharged there was no difference in length of stay in the intensive treatment unit (median 5 (range 0–24) *versus* 5 (range 0–84) days) or in-hospital stay (median 66 (range 29–159) *versus* 75 (range 31–202) days) between the two time periods.

Conclusion: A minimally invasive approach provides a promising alternative to open pancreatic necrosectomy.

Role of a spiral computed tomographic grading system in increasing the efficacy of laparoscopic staging for potentially resectable pancreatic and periampullary tumours

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Background: In a recent review of patients with potentially resectable pancreatic malignancy in the authors' unit, it was shown that inappropriate laparotomy was avoided in 35 per cent of patients as a direct result of laparoscopy combined with laparoscopic ultrasonographic staging, following previous spiral computed tomography (CT). However, subsequent analysis has suggested that laparoscopy may have added to information already available on the CT scan in as few as 22 per cent of patients. The aim of this study was to seek to define spiral CT scan criteria that might provide a more selective approach to laparoscopic staging.

Methods: Theatre records and the Lothian Surgical Audit Database were used to identify 124 patients between June 1995 and May 2000 whose investigations for suspected pancreatic tumours had included spiral CT followed by laparoscopic assessment (suspected tumour: pancreatic head, 88; periampullary, 25; lower bile duct, nine; duodenum, two). Each CT scan (with endoscopic retrograde cholangiopancreatography films if appropriate) was examined by a specialist pancreaticobiliary radiologist (D.N.R.) and graded 'blindly' according to a modified existing system for determining local resectability (0, no mass; A, fat plane between tumour and vessels; B, normal pancreas between tumour and vessels; C, mass convex to vessel; D, mass concave to vessel; E, vessel encircled; F, vessel occlusion) before the results were compared with findings at laparoscopy and laparotomy.

Results:

	0	A	B	C	D	E	F
n	27	24	4	8	29	28	4
Resection	20	9	3	1	9	—	—
Irresectable							
Laparoscopy	2	3	—	—	3	2	—
Laparoscopy + laparoscopic US	1	—	—	1	4	2	2
Laparoscopic US	—	5	—	1	8	11	2
No laparotomy (other)	1	1	—	2	1	1	—
Inappropriate laparotomy	3	6	1	3	4	12	—

US, ultrasonography

In groups 0, E and F, only four (7 per cent) of 59 patients benefited from laparoscopic assessment, whereas 27 (42 per cent) of 65 patients avoided an inappropriate laparotomy in groups A–D as a result of improved staging.

Conclusion: Based on these results, only patients graded A–D should undergo laparoscopic staging. By adopting this more selective policy in the future, the number of laparoscopic evaluations may be reduced by 48 per cent, while maintaining the efficacy of preventing inappropriate laparotomies at 42 per cent.

Role of hepatectomy in the management of bile duct injuries

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Background: Laparoscopic cholecystectomy is associated with bile duct injuries of a more severe nature than open cholecystectomy. This study examined the emerging role of hepatectomy in the management of major iatrogenic bile duct injuries in the laparoscopic era.
Methods: This was a retrospective cohort study of patients referred to a tertiary hepatobiliary unit with bile duct injuries over a 16-year period until April 2000. Data are expressed as median (range).
Results: Eighty-eight patients (34 men, 54 women) were referred during this interval; their median age was 55 (19–83) years. Injuries resulted from 50 laparoscopic cholecystectomies and 35 open cholecystectomies, with three occurring during gastroduodenal procedures. Laparoscopic surgery was associated with injuries of greater severity than open cholecystectomy: Bismuth type I–II, 32 per cent *versus* 69 per cent for the open operation; type III–IV, 66 per cent *versus* 31 per cent for the open procedure ($P = 0{\cdot}02$, χ^2 test). After referral 73 patients underwent definitive surgical interventions: 57 hepaticojejunostomies, 11 revisions of hepaticojejunostomy, two orthotopic liver transplants and three right hepatectomies. Two patients had subsequent hepatectomy following initial hepaticojejunostomy. Four of the five hepatectomies were for the management of injuries perpetrated at laparoscopic cholecystectomy. Criteria necessitating hepatectomy were liver atrophy on computed tomography (80 *versus* 11 per cent; $P = 0{\cdot}0001$, χ^2 test) and a greater incidence of angiographically proven vascular injury (40 *versus* 6 per cent; $P = 0{\cdot}006$, χ^2 test); in addition, type III–IV injuries were more frequent (60 *versus* 42 per cent) in the hepatectomy group. There were no procedure-related deaths. The overall postoperative morbidity rate was 13 per cent. Median hospital stay was 10 days.
Conclusion: Major hepatectomy allows the successful and safe repair of cholecystectomy-related bile duct injuries complicated by concomitant vascular injury, unilateral lobar atrophy and destruction of the biliary confluence.

New classification of oesophageal and gastric carcinomas

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Background: The current International Classification of Diseases (ICD)-O classification of carcinomas of the oesophagus and stomach causes epidemiological and clinical confusion, particularly the use of the term cardia and the overlapping subsites described in the stomach. This study compared the epidemiological and clinical features of each subtype and subsite of carcinoma of the oesophagus and stomach to assess requirements for a new classification of these carcinomas.
Methods: Data were extracted with appropriate validity checks on all cases of oesophageal and gastric carcinoma identified throughout the period 1974–1993 by the Merseyside and Cheshire Cancer Registry, which covers a population of 2·5 million. Comparison of all identifiable epidemiological and clinical features of adenocarcinomas at four different subsites, namely the upper two-thirds of the oesophagus, the lower third of the oesophagus, cardia and subcardia of the stomach, was performed.
Results: There were 5322 primary carcinomas of the oesophagus and 10 535 carcinomas of the stomach registered between 1974 and 1993. The incidence of adenocarcinoma of the lower oesophagus and cardia trebled in males and doubled in females, whereas adenocarcinoma of the subcardia region of the stomach declined in both sexes. The incidence of adenocarcinoma of the lower oesophagus and of the cardia was similar for median age at diagnosis, male : female ratio, percentage of patients who smoked, and survival; both were significantly different from values for carcinoma of the subcardia in these respects.
Conclusion: These data suggest that there is considerable overlap between adenocarcinomas of the lower oesophagus and adenocarcinomas currently classified as of the cardia. The authors believe this is due to the group of carcinomas classified as cardia consisting mainly of carcinomas that traverse the gastro-oesophageal junction. These carcinomas were different in all studied parameters from carcinomas of the stomach and should be classified separately from gastric carcinomas. A new subsite classification of oesophageal and gastric carcinomas is proposed that includes the gastro-oesophageal junction as a subsite of the oesophagus and that simplifies the subsite classification of the stomach into proximal, distal and overlapping.

Neoadjuvant chemoradiotherapy for operable oesophageal carcinoma: preliminary results from Sheffield

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Background: Surgical resection is the mainstay of treatment for potentially curable oesophageal carcinoma but the long-term survival rate remains 10–20 per cent. Neoadjuvant administration of chemoradiotherapy (NCR) may improve these values. In this study the authors reviewed their preliminary experience with NCR in Sheffield.
Methods: Twenty-five patients with potentially resectable oesophageal carcinoma embarked on a regimen of NCR, with resection planned 4–6 weeks later. Chemotherapy incorporated two cycles of intravenous *cis*-platinum and 5-fluorouracil with external-beam radiotherapy administered synchronously (30–45 Gy).
Results: Twenty-two of the 25 patients suffered side-effects from NCR, including one death, and seven patients failed to complete NCR as planned. The median interval from diagnosis to surgery was 121 days. Twelve out of 24 patients had significant postoperative complications, including two deaths Seven patients had a complete histological response to NCR

(three out of 15 for adenocarcinoma, four out of nine for squamous carcinoma).

Conclusion: The complete histological response rate to NCR in these patients compares favourably with previous studies, as does the postoperative mortality, but this was at the expense of substantial morbidity and was associated with long delays from diagnosis to operation. At present it is not possible to predict which patients will respond favourably to NCR and whether they will benefit with improved survival.

Magenstrasse and Mill vertical gastric stapling for morbid obesity

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Background: The aim of the study was to determine the effects of a new vertical gastric stapling procedure used alone or in combination with a Roux-en-Y gastric bypass for patients with morbid obesity.

Methods: A consecutive series of 100 patients with morbid obesity underwent either vertical gastric stapling alone (Magenstrasse and Mill procedure; MM) ($n = 80$) or combined with a limited Roux-en-Y gastric bypass (MM-GB) ($n = 20$). Median age (39 *versus* 40 years), body mass index (45·9 *versus* 45·8) and M : F ratio (1 : 4 *versus* 1 : 3) were similar between the two groups of patients. The median follow-up of patients who underwent the MM procedure was 36 (range 0–72) months, and 15 (0–36) months for patients who had the combined MM-GB procedure.

Results: The operative mortality rate was 0 per cent and there were no major postoperataive complications. Median length of stay was 7 days for patients who had the MM procedure and 10 days for those who underwent combined MM-GB. One year after operation median(s.d.) weight loss was 38·6(15·1) kg in the MM group, compared with 50·4(8·4) kg in the MM-GB group ($P = 0{\cdot}02$). Patients who had the MM procedure had lost 30(8·3) per cent of their original weight, compared with 36(3·2) per cent for those who had a combined MM-GB procedure ($P = 0{\cdot}03$).

Conclusion: Gastric stapling for morbid obesity is safe in specialized surgical units. Weight loss after the combined MM-GB procedure was greater than that following the MM procedure alone, although this has to be set against the potential long-term complications of a gastric bypass procedure.

Laparoscopic Nissen *versus* anterior fundoplication in patients with oesophageal dysmotility: a randomized controlled trial

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Background: It has been postulated that in patients with oesophageal dysmotility a partial fundoplication may be associated with a better outcome than a Nissen fundoplication. This study aimed to compare laparoscopic anterior fundoplication with laparoscopic Nissen fundoplication in patients with disordered peristalsis.

Methods: One hundred and seven patients with documented gastro-oesophageal reflux disease were randomized to undergo either a Nissen fundoplication ($n = 53$) or an anterior 180° hemifundoplication ($n = 54$), and were seen at follow-up at least once a year by a blinded investigator who used standardized clinical grading systems to assess postoperative dysphagia, heartburn and patient satisfaction. Patients were divided into two groups depending on preoperative oesophageal manometric findings: normal peristalsis (81 patients) or disordered peristalsis (26 patients – propagated less than 50 per cent of swallows and/or distal peristaltic amplitude less than 40 mmHg).

Results: At all follow-up intervals beyond 6 months, patients who underwent an anterior fundoplication experienced less dysphagia and were more likely to be satisfied with the clinical outcome. The magnitude of outcome differences was independent of peristaltic status, and there was no trend towards a poorer outcome in patients with poor peristalsis who underwent Nissen fundoplication.

Conclusion: The concept of tailoring the specific antireflux procedure according to preoperative peristaltic status was not supported by this trial. However, laparoscopic anterior fundoplication was associated with less dysphagia overall and an improved clinical outcome at 3 years.

Photodynamic therapy for dysplastic Barrett's oesophagus: long-term follow-up

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Background: Photodynamic therapy (PDT) is a novel treatment in which tissue damage is caused by the action of light on a previously administered photosensitizing agent. The results of long-term follow-up of the use of PDT in patients with dysplastic Barrett's oesophagus are presented.

Methods: Thirty-seven patients with low-grade dysplastic Barrett's oesophagus were treated with oral 5-aminolaevulinic acid (ALA) 30 mg kg^{1}, followed by laser endoscopy 4 h later. Patients were treated between December 1995 and December 1998, and all were followed regularly with endoscopy and biopsies in the authors' surveillance programme.

Results: All patients initially showed eradication of dysplasia, and 32 of 37 had a macroscopic reduction in the length of columnar epithelium. The effect was maintained for a median of 43 (range 18–54) months, although one patient developed a late carcinoma.

Conclusion: ALA-induced PDT can provide safe and effective ablation of low-grade dysplastic Barrett's oesophagus, and the effects appear to be maintained for a protracted period of time.

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